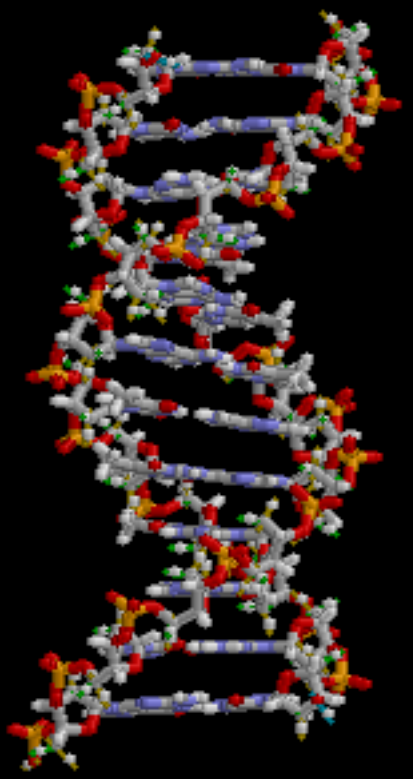
# DNA

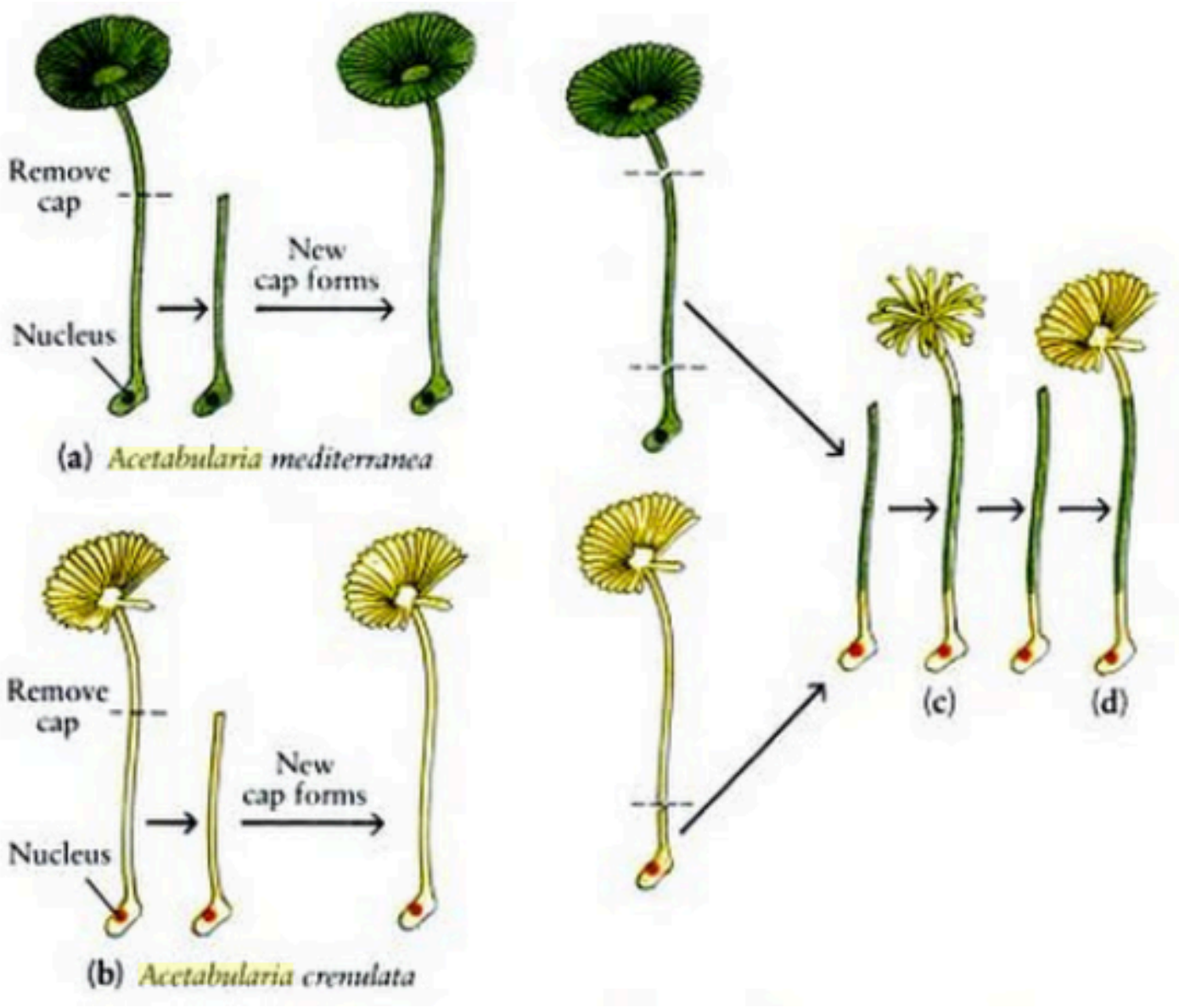
Its discovery, structure, and replication

# Essential Questions from DNA Discovery Activity

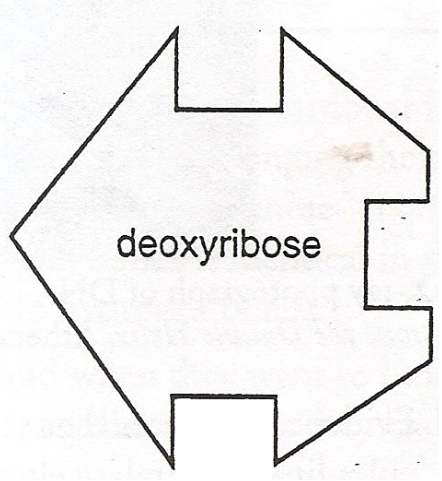
1. How do we know that DNA is the genetic material?
2. What's the evidence for the current model of DNA

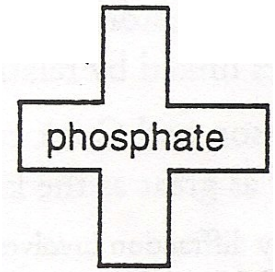


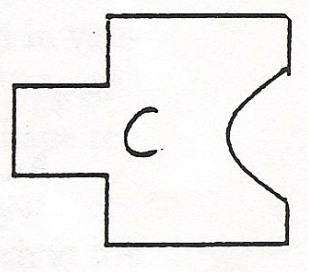
# Significance of *Acetabularia*

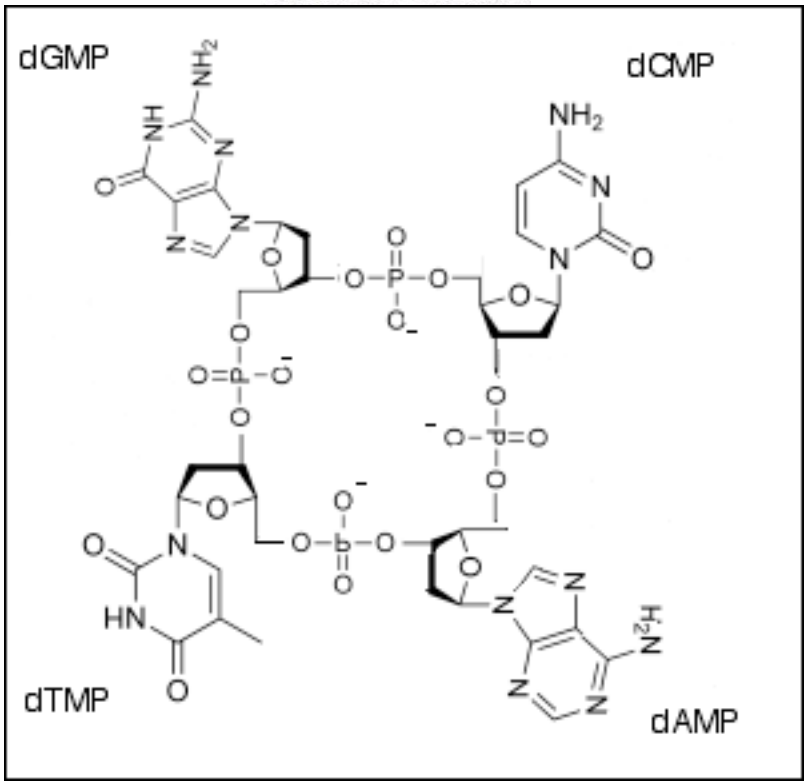


# *Nuclein*: Known chemistry…wrong ideas



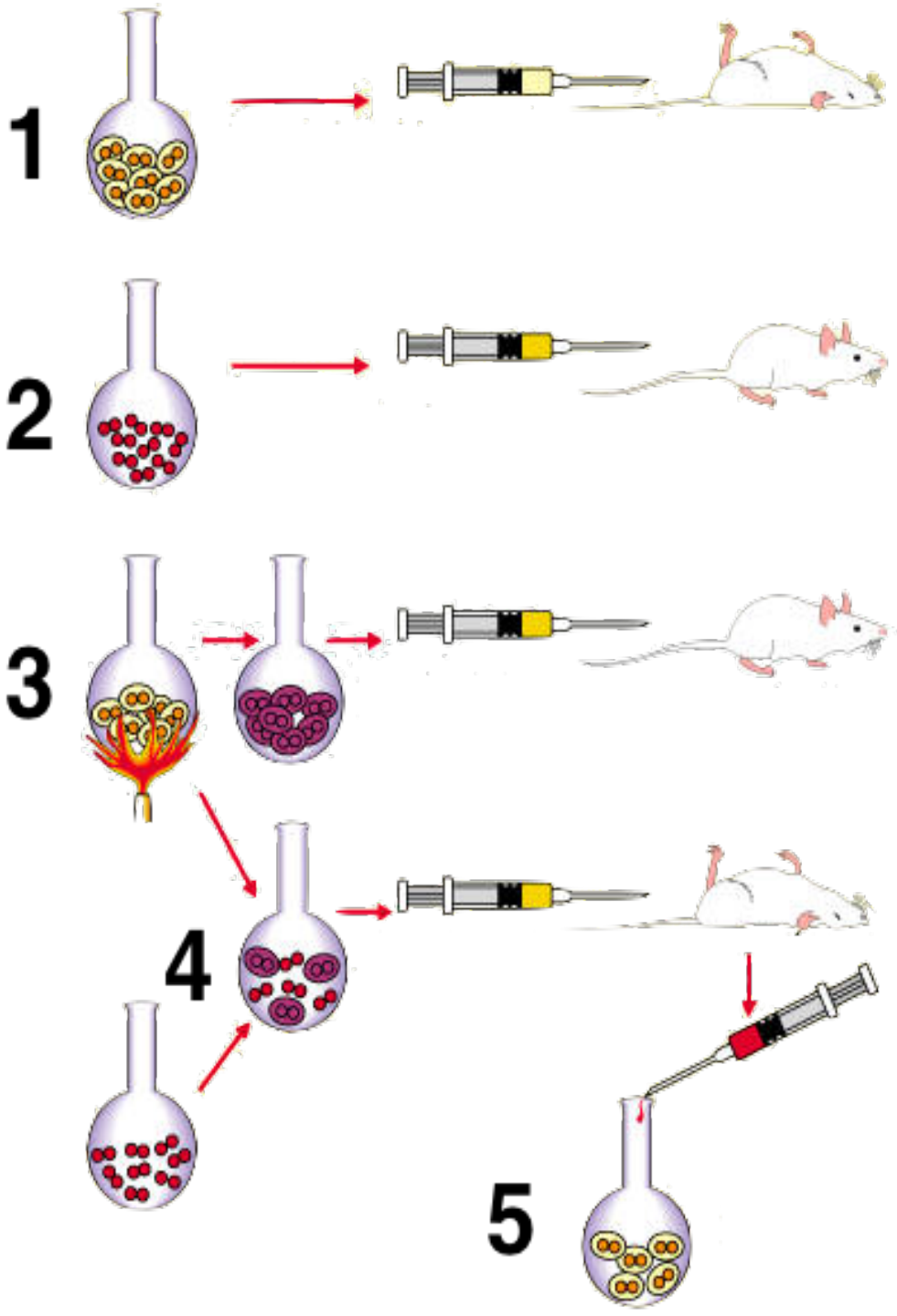



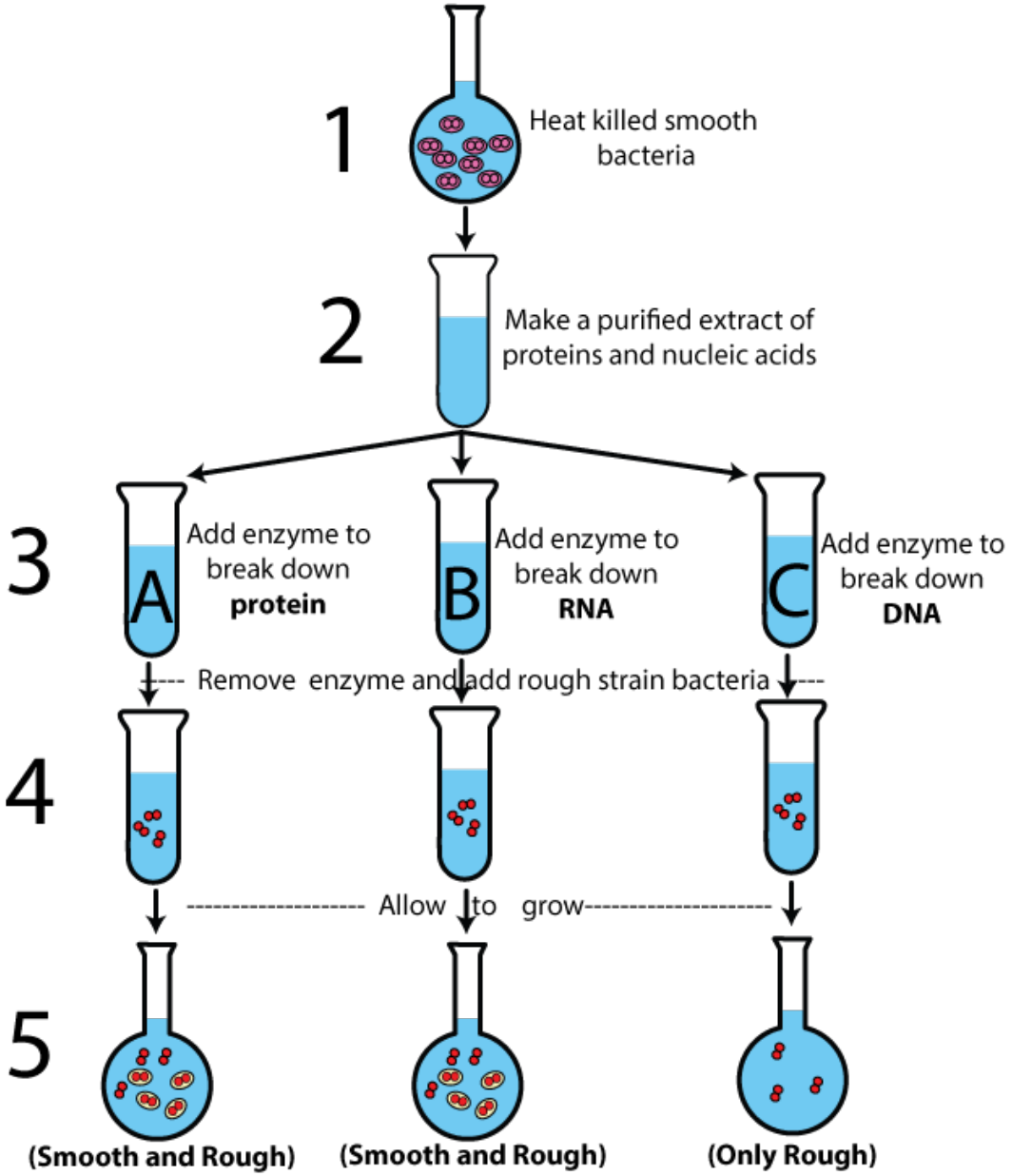




Tetra-nucleotide hypothesis

# The *Transforming Factor* Experiment

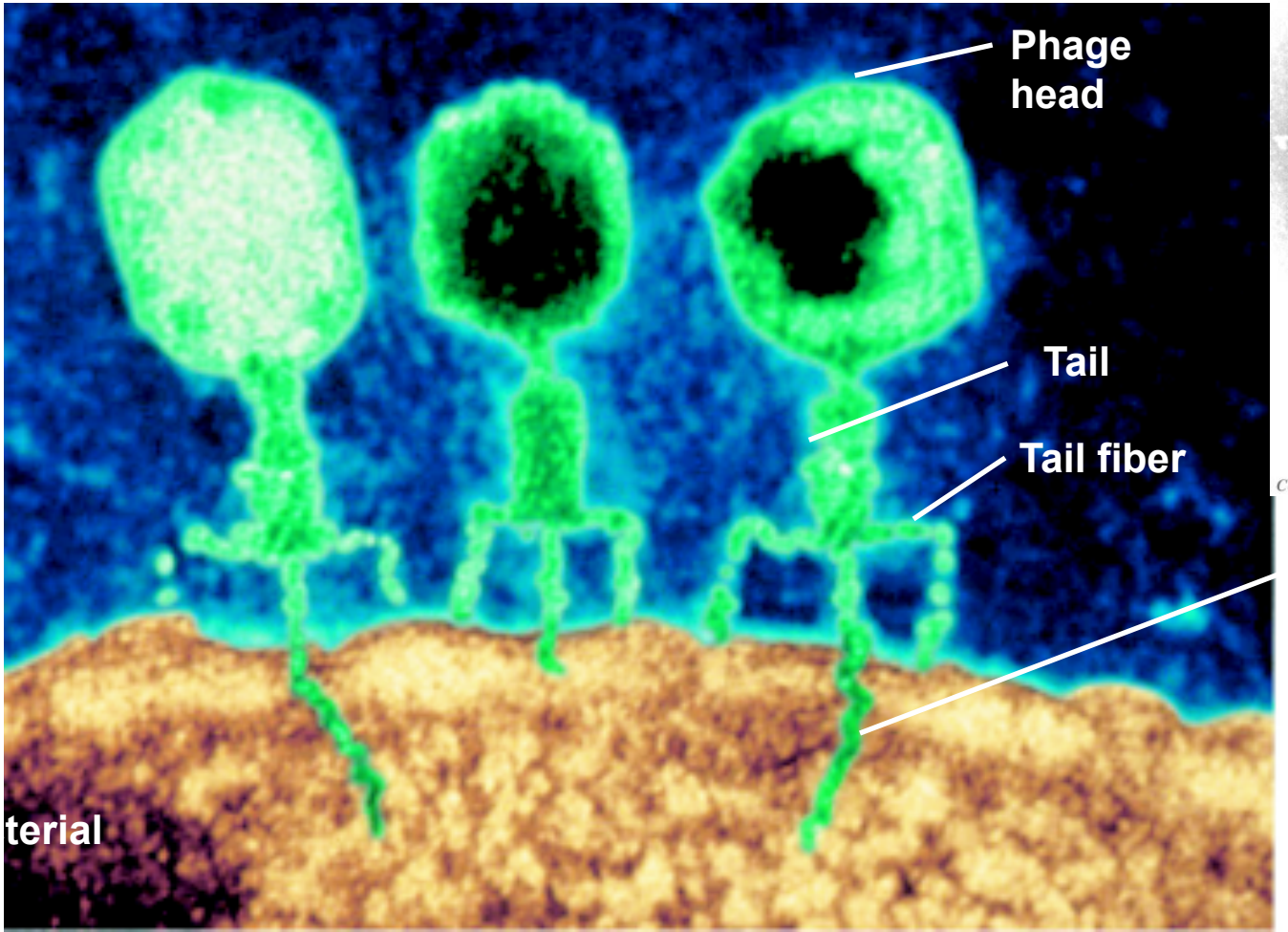


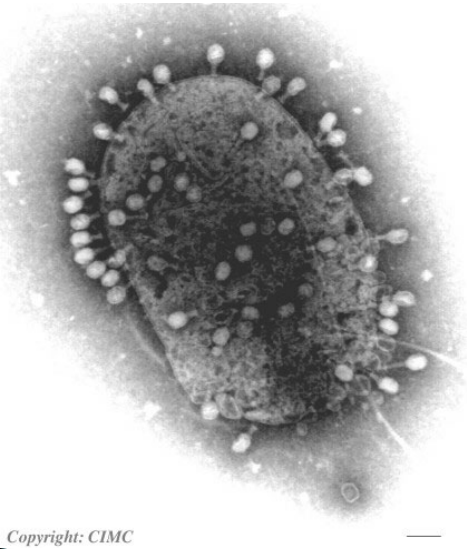
# Identifying the Transforming Factor




1. If you break down the DNA, you don't get _____ to occur.
2. Therefore, ____ must be the Transforming Factor

# Hershey Chase Experiment: Phage attacking a bacterial cell

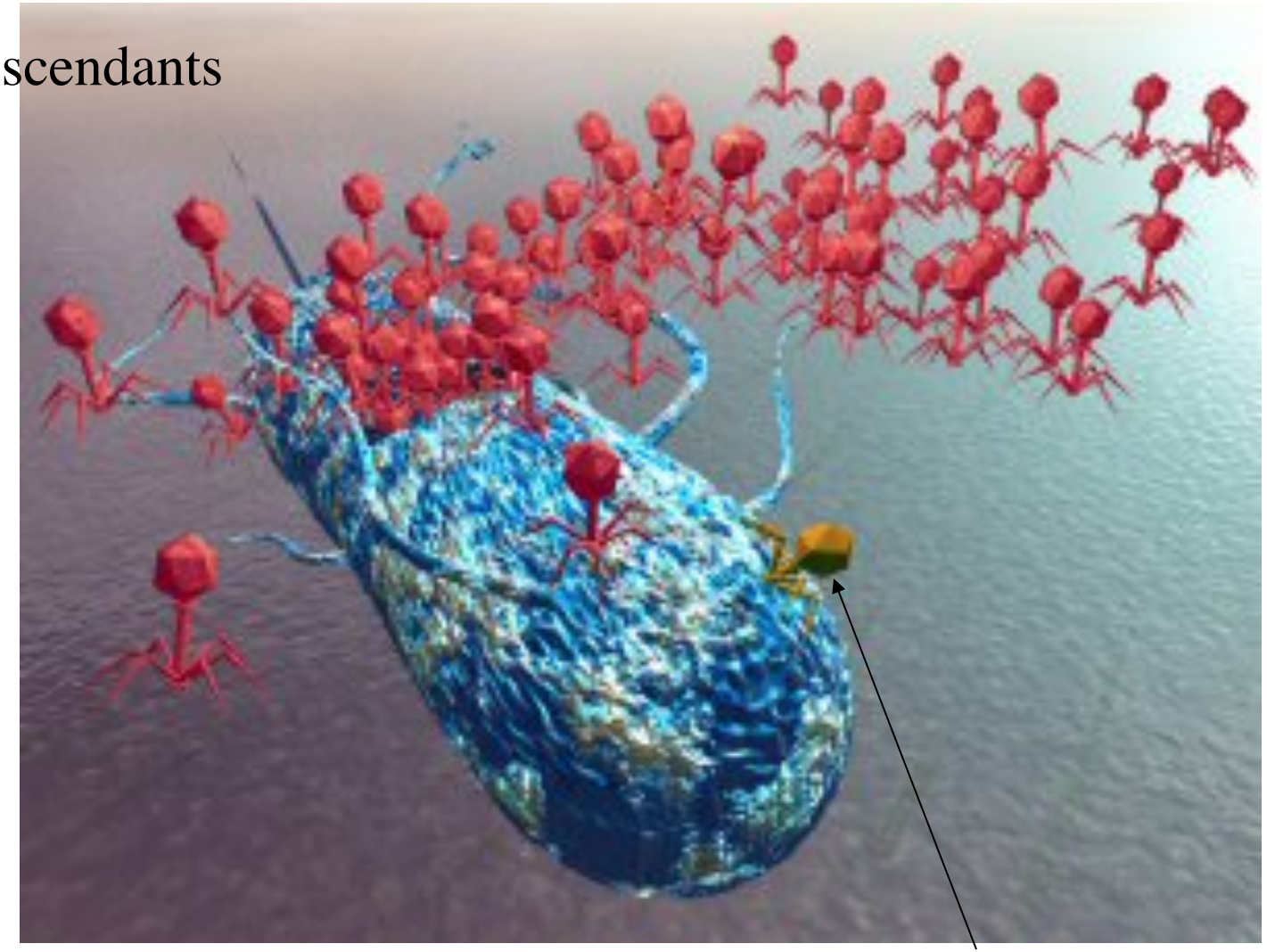


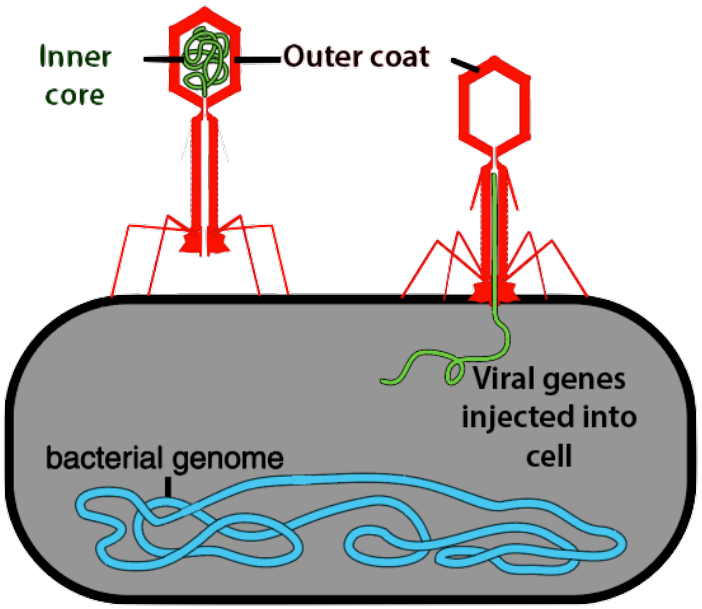

**What are these viruses injecting into the cell?**

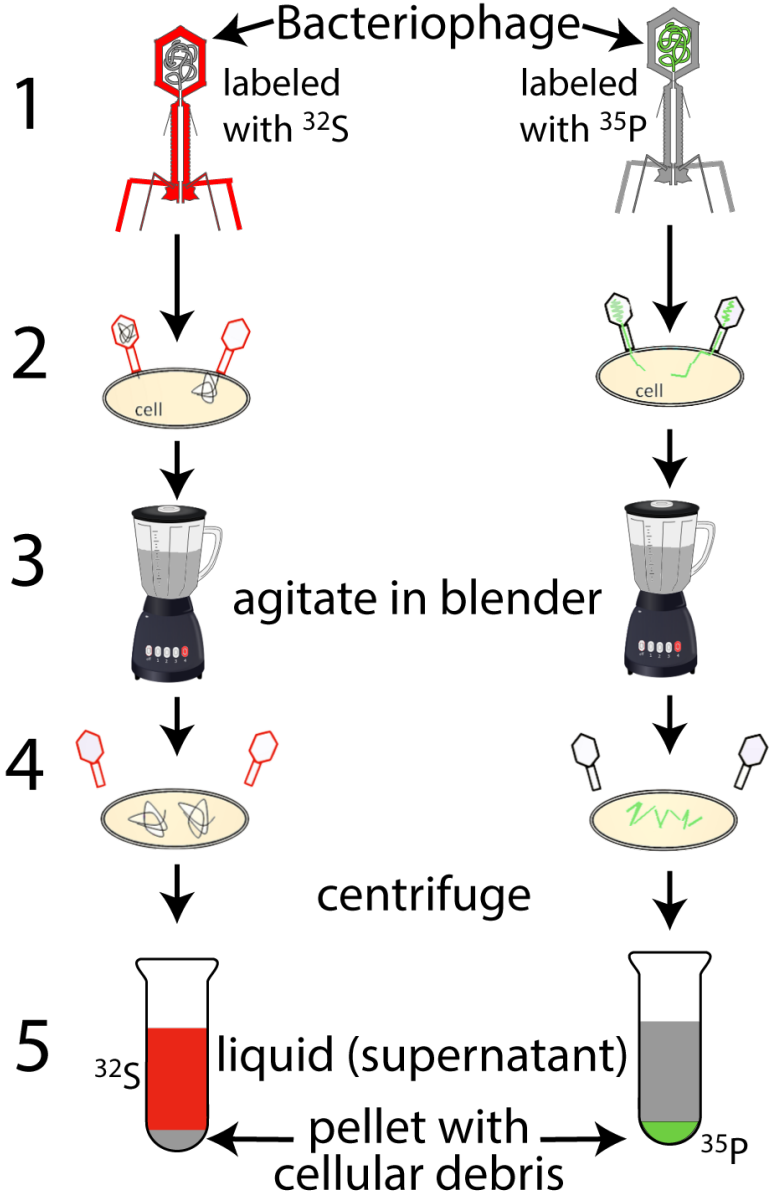
100 nm

# The result of phage attack



# Hershey Chase Experiment



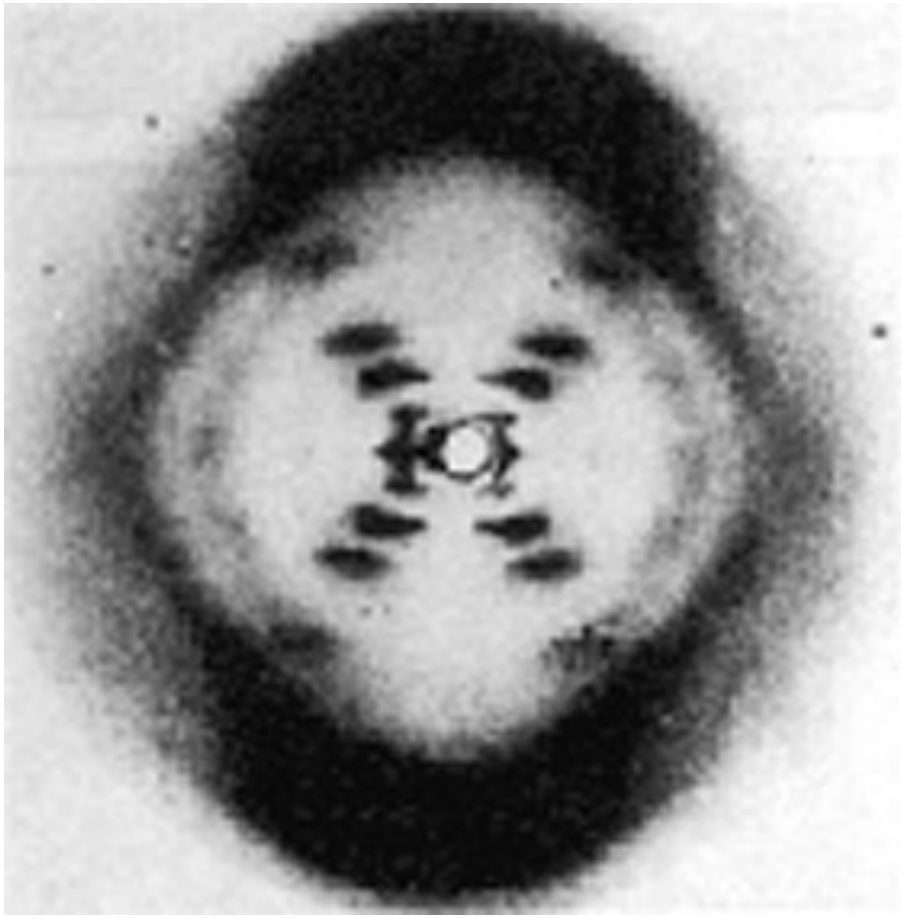
# So, the race was on to figure out the structure of DNA

Linus Pauling: discover of the structure of hemoglobin and cause of sickle cell anemia

Rosalind Franklin: molecular biologist doing X-Ray studies of DNA with Maurice Wilkins

James Watson (American biologist) and Francis Crick (British Physicist)

# Franklin's X-Ray Diffraction Image of DNA says that DNA has a helical structure, and that it's about 2nm wide



# *Also, Chargaff's Rule: Relative Proportions (%) of Bases in DNA*

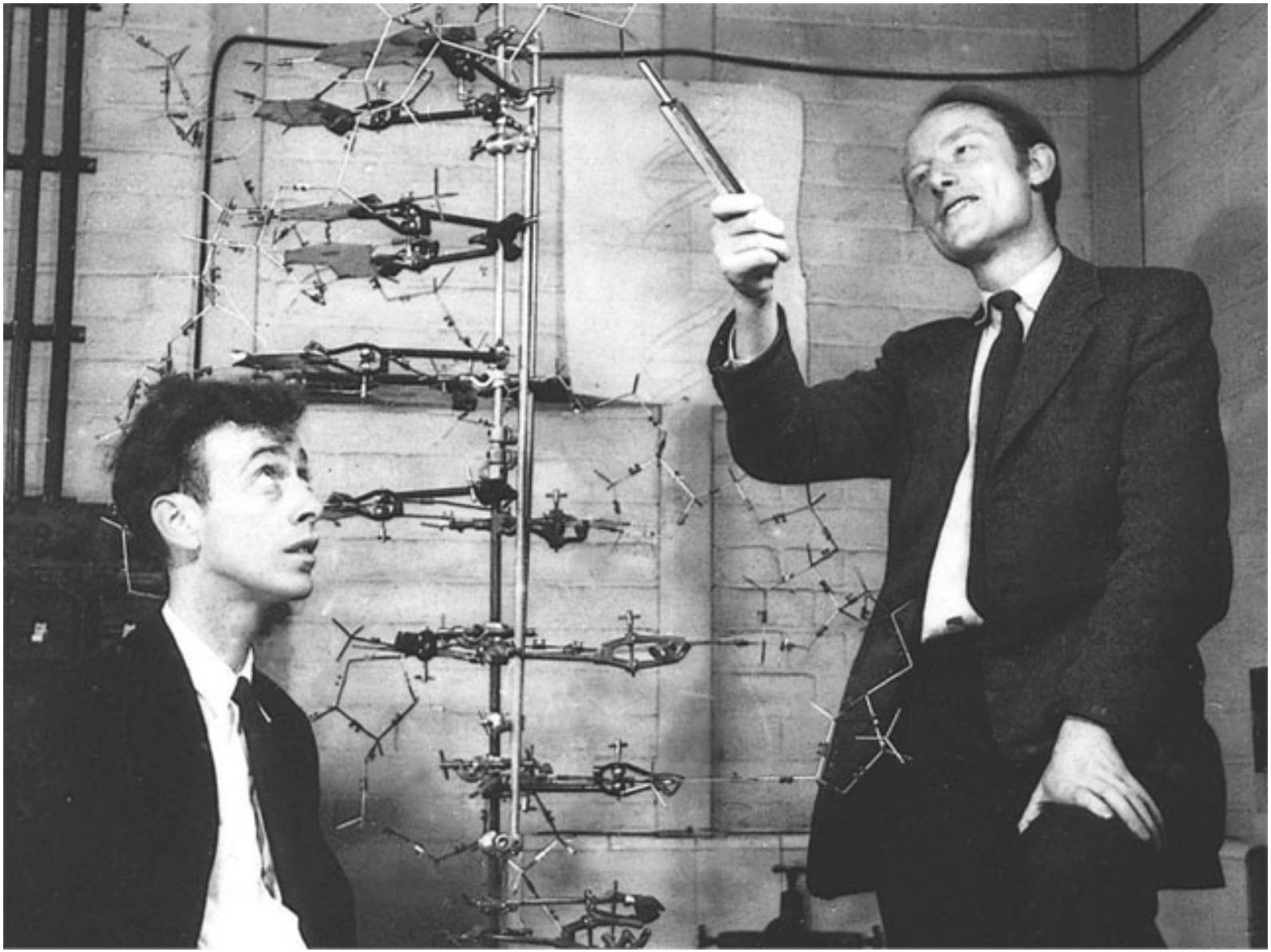
| Organism | %A | %G | %C | %T |
|---|---|---|---|---|
| Maize | 26.8 | 22.8 | 23.2 | 27.2 |
| Octopus | 33.2 | 17.6 | 17.6 | 31.6 |
| Chicken | 28.0 | 22.0 | 21.6 | 28.4 |
| Rat | 28.6 | 21.4 | 20.5 | 28.4 |
| Human | 29.3 | 20.7 | 20.0 | 30.0 |
| Grasshopper | 29.3 | 20.5 | 20.7 | 29.3 |
| Sea Urchin | 32.8 | 17.7 | 17.3 | 32.1 |
| Wheat | 27.3 | 22.7 | 22.8 | 27.1 |
| Yeast | 31.3 | 18.7 | 17.1 | 32.9 |
| E. coli | 24.7 | 26.0 | 25.7 | 23.6 |

*Keeping in mind that scientific measurements always have some margin of error, what relationship do you notice among the bases?*

# The last clues

- An adenine-thymine pair has the same width as a cytosine-guanine pair.
- Each of these pairs is about 2nm wide (the same width as a DNA molecule…

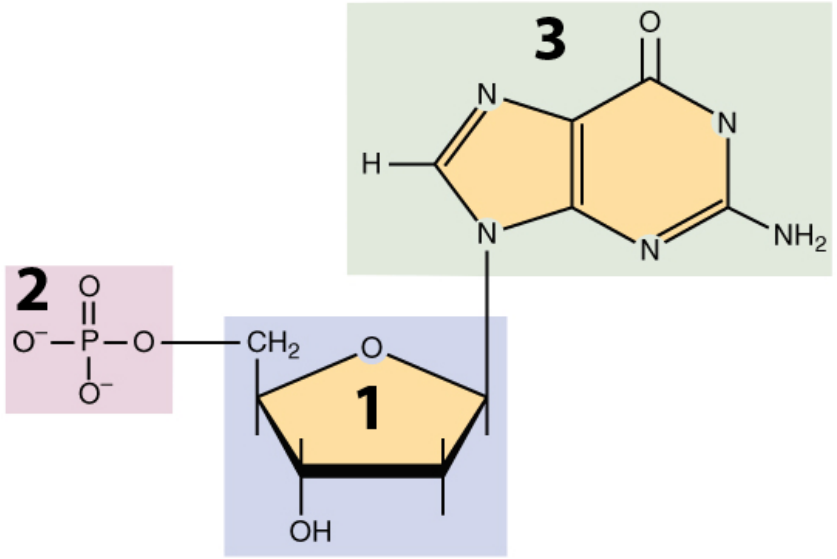
# Watson and Crick Model of DNA

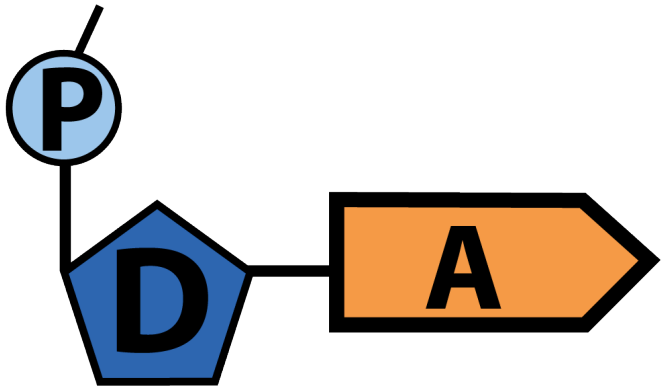


# What you need to know about DNA

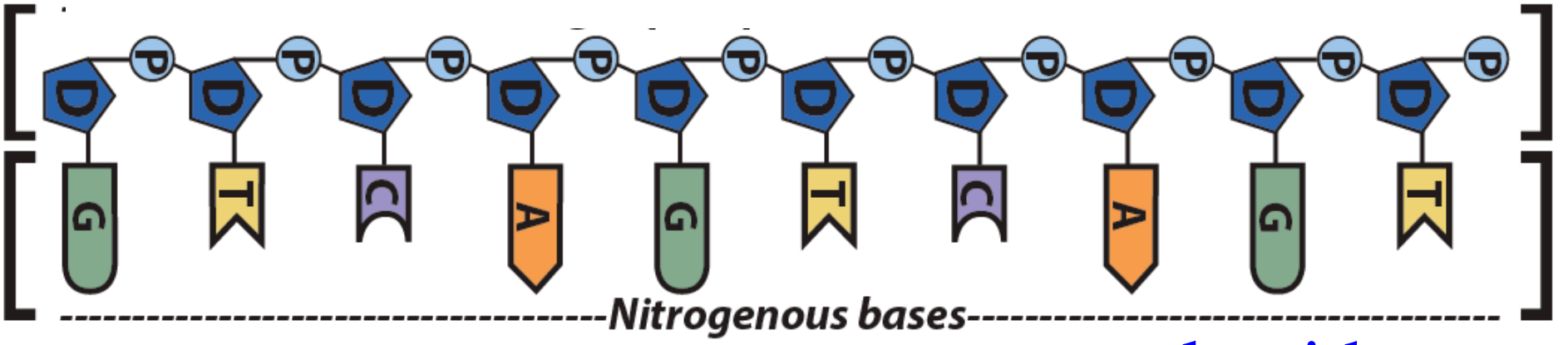
Monomer is a *nucleotide*

- *Deoxyribose (sugar)*
- *Phosphate*
- One of four *nitrogenous* bases
  - –***A***denine
  - –***T***hymine
  - –***C***ytosine
  - –***G***uanine



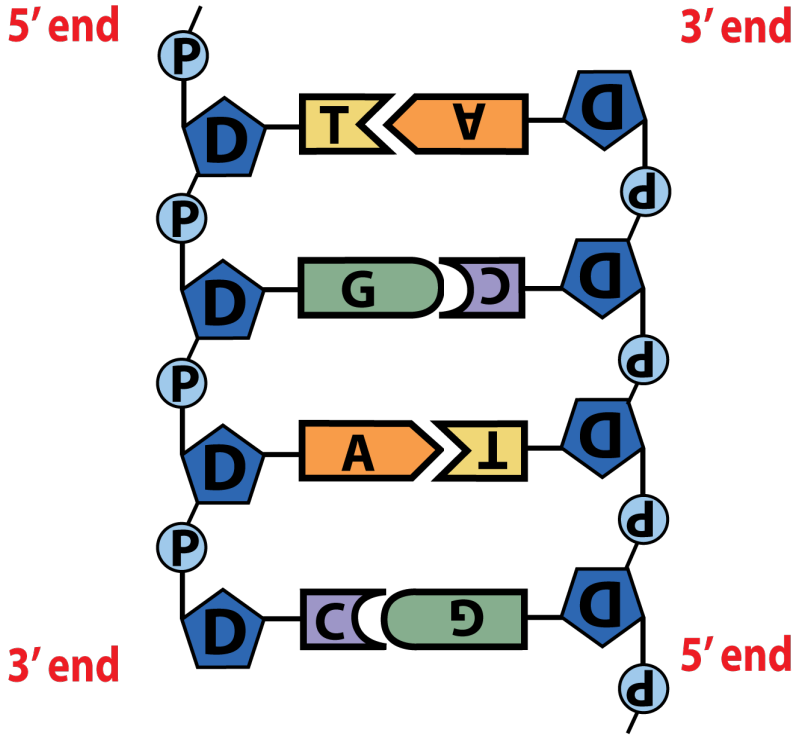



# A single strand



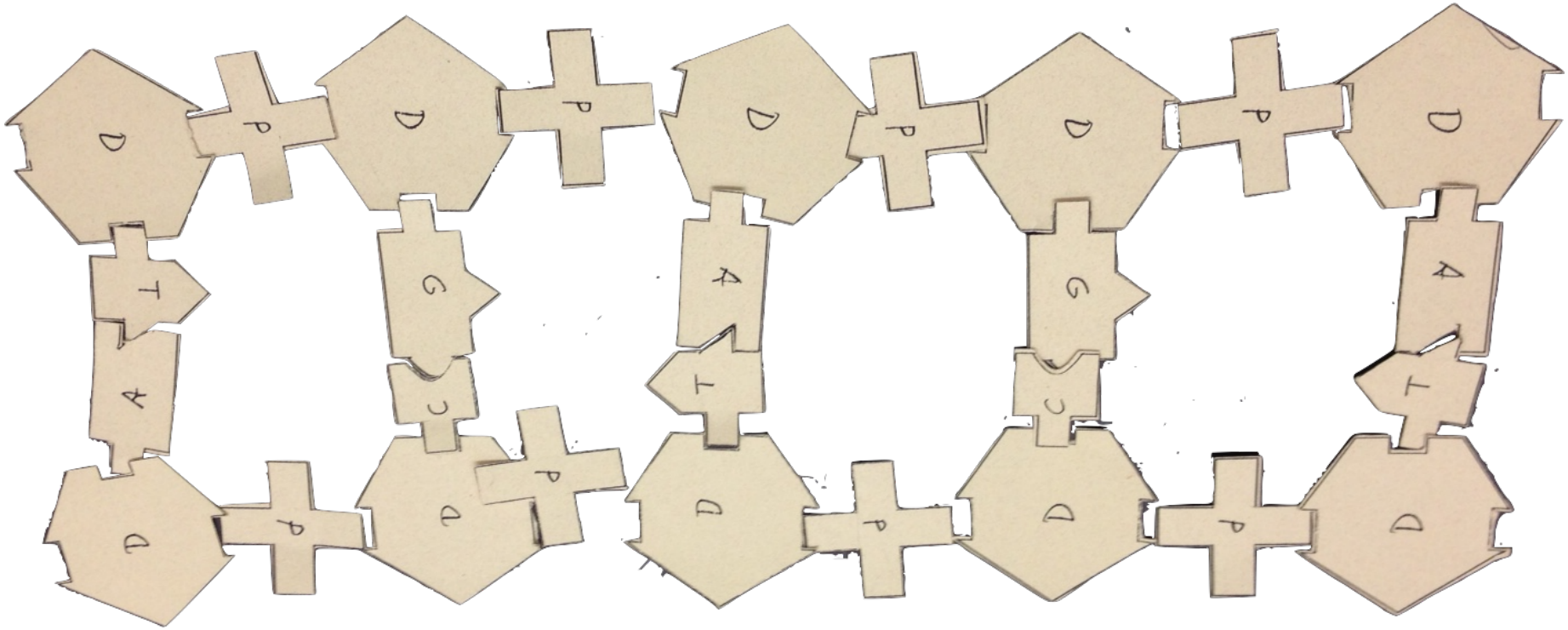

- A single strand consists of nucleotides chained together by covalent bonds.
- This creates a sugar - phosphate backbone
- Genetic information is stored in the sequence of bases.

# Overall structure is ___double___ stranded



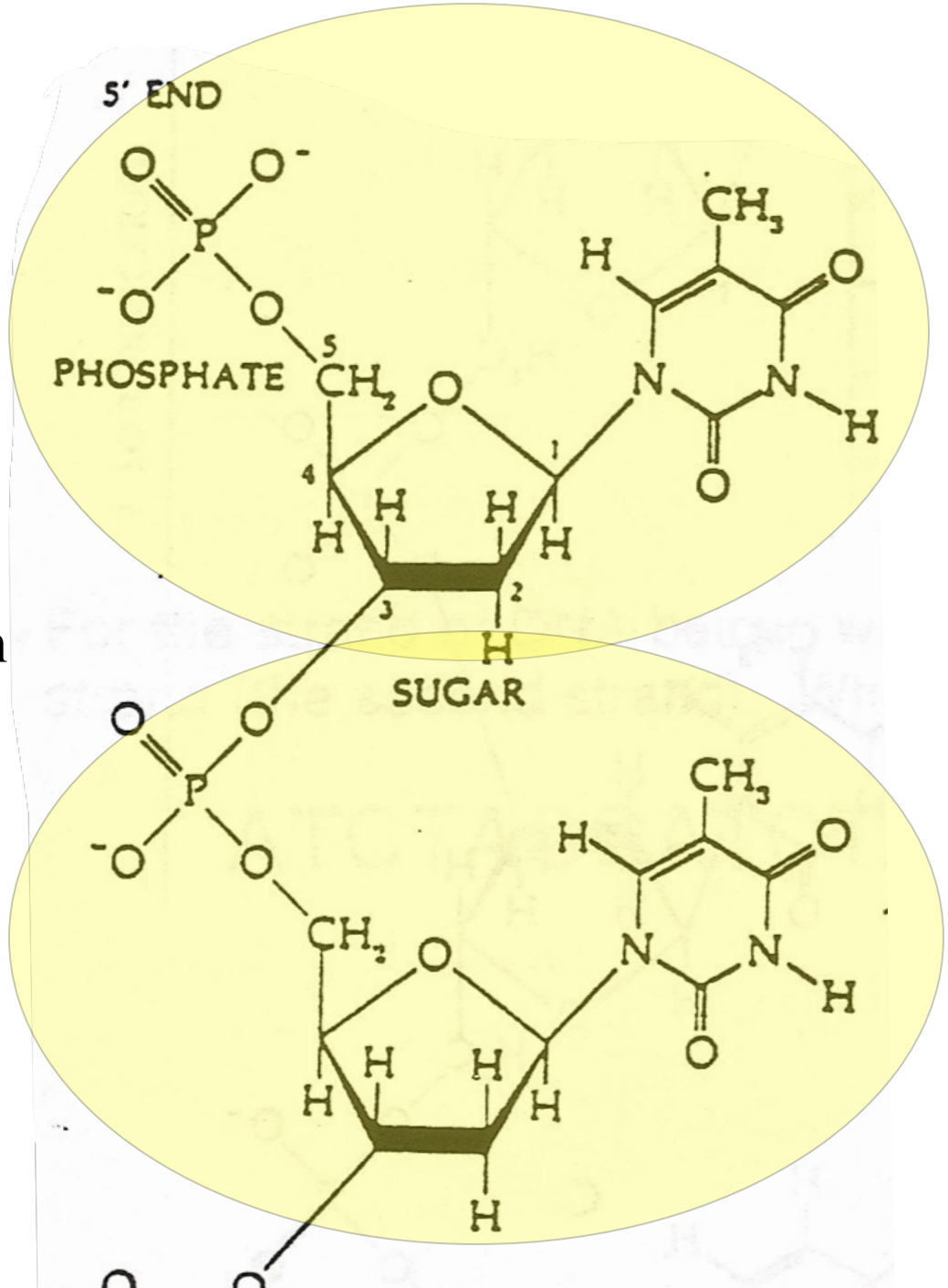

- If DNA were a ladder, the nitrogenous bases would form the ___rungs___. The sides would consist of ___sugars___ and ___phosphates___.
- The shape of A ___complements___ the shape of T. Same for G and ___C___.
- For the strands to bind, they have to be ___anti___ -___parallel___.
- ___Hydrogen___ bonds hold the bases together.
- The structure allows for accurate ___replication___ and transfer of ___information___

# Not parallel, but *anti-parallel*



# DNA has *directionality*

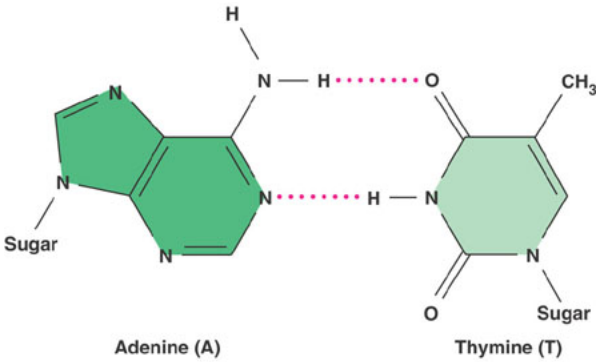
- Each nucleotide's **phosphate** is attached to the **5'** carbon in deoxyribose
- When DNA is being synthesized, **enzymes** can only add new nucleotides at the **3'** end.



# Base Pairing Rules

A

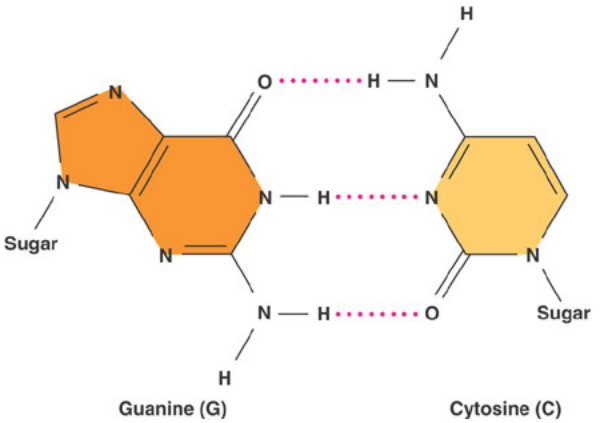
Two-ring bases are ***purines***




T

One ring bases are ***pyrimidines***

G

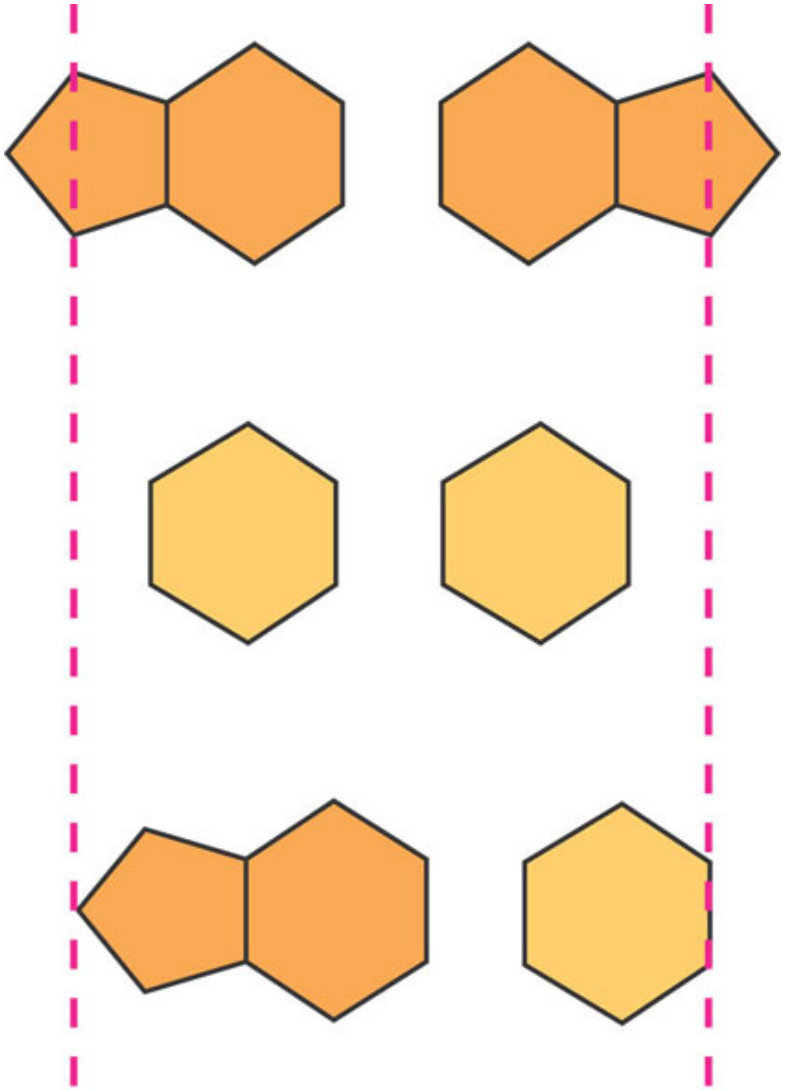



C

- Note that the hydrogen bonds are between H and either ***nitrogen*** or ***oxygen*** on the opposing base.
- A purine always bonds with a ***pyrimidine***

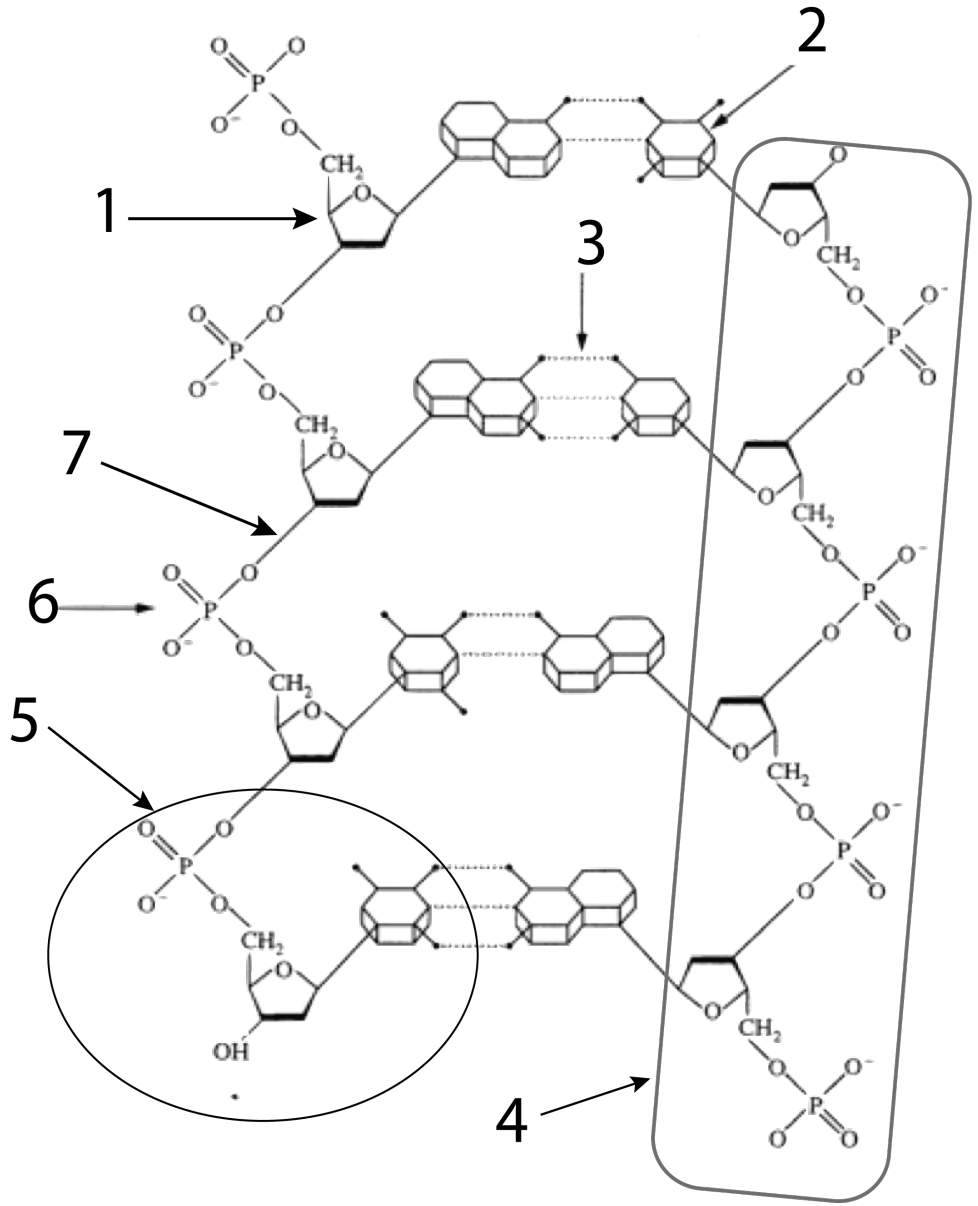
# Base pairing rules

(no writing needed)



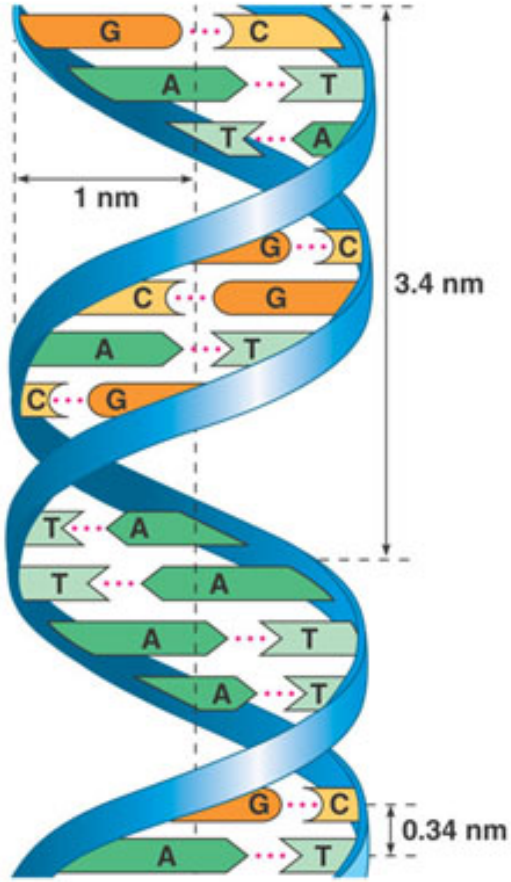
- Two purines : too wide
- Two pyrimidines : too narrow
- A purine and a pyrimidine: just right!

# Checking Understanding



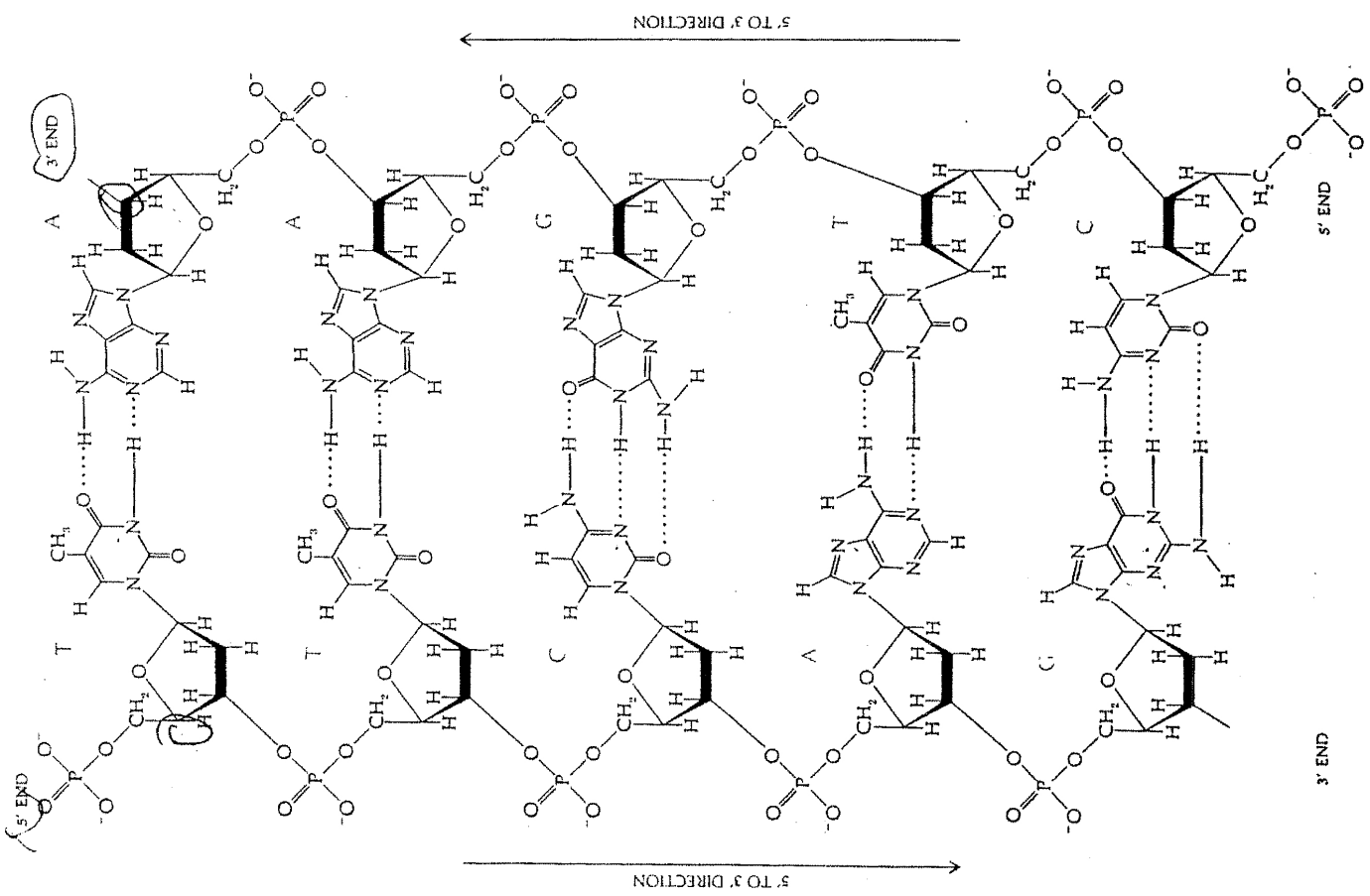

1. Deoxyribose
2. Nitrogenous base
3. Hydrogen bond
4. Sugar-phosphate backbone
5. Nucleotide
6. Phosphate group
7. Sugar-phosphate bond

# DNA's dimensions



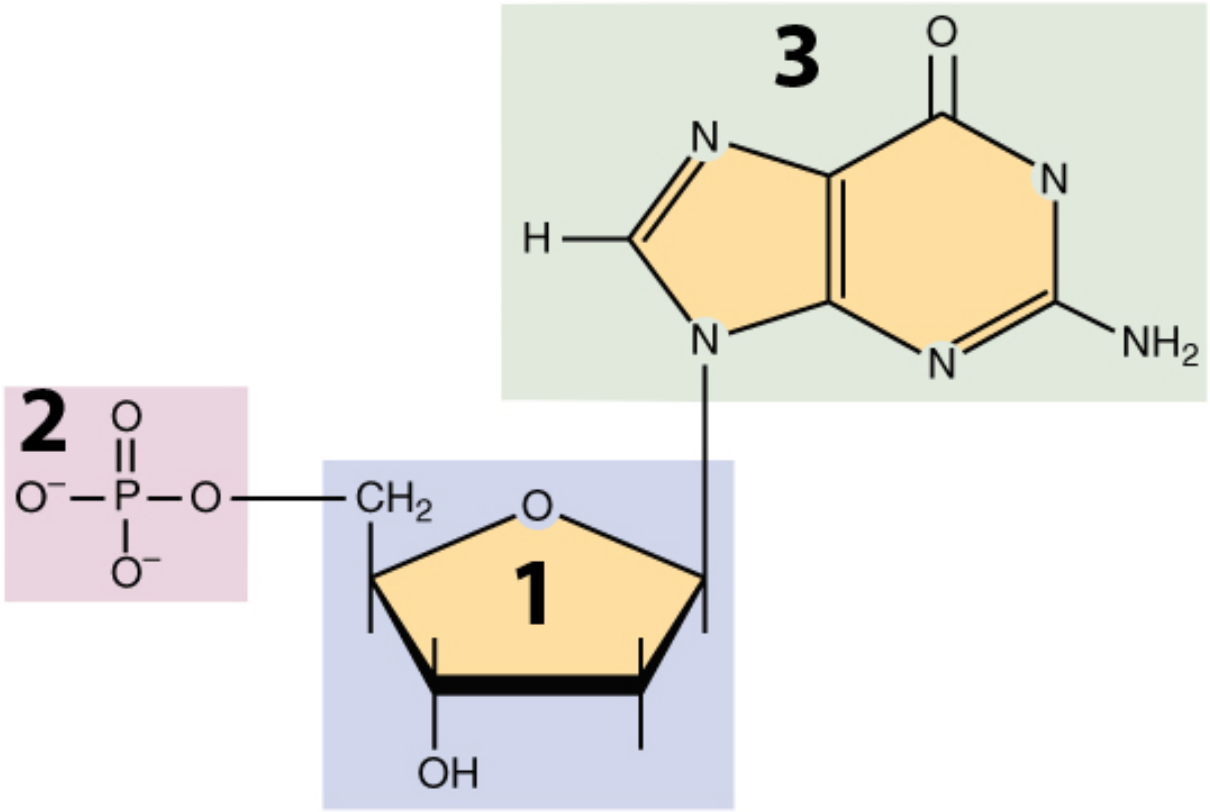
(a) Key features of DNA structure

- Length is *indeterminate*
- Diameter is *2* nanometers
- Adjacent bases are *.34* nm apart
- *10* bases make one turn, and a turn is *3.4* nm

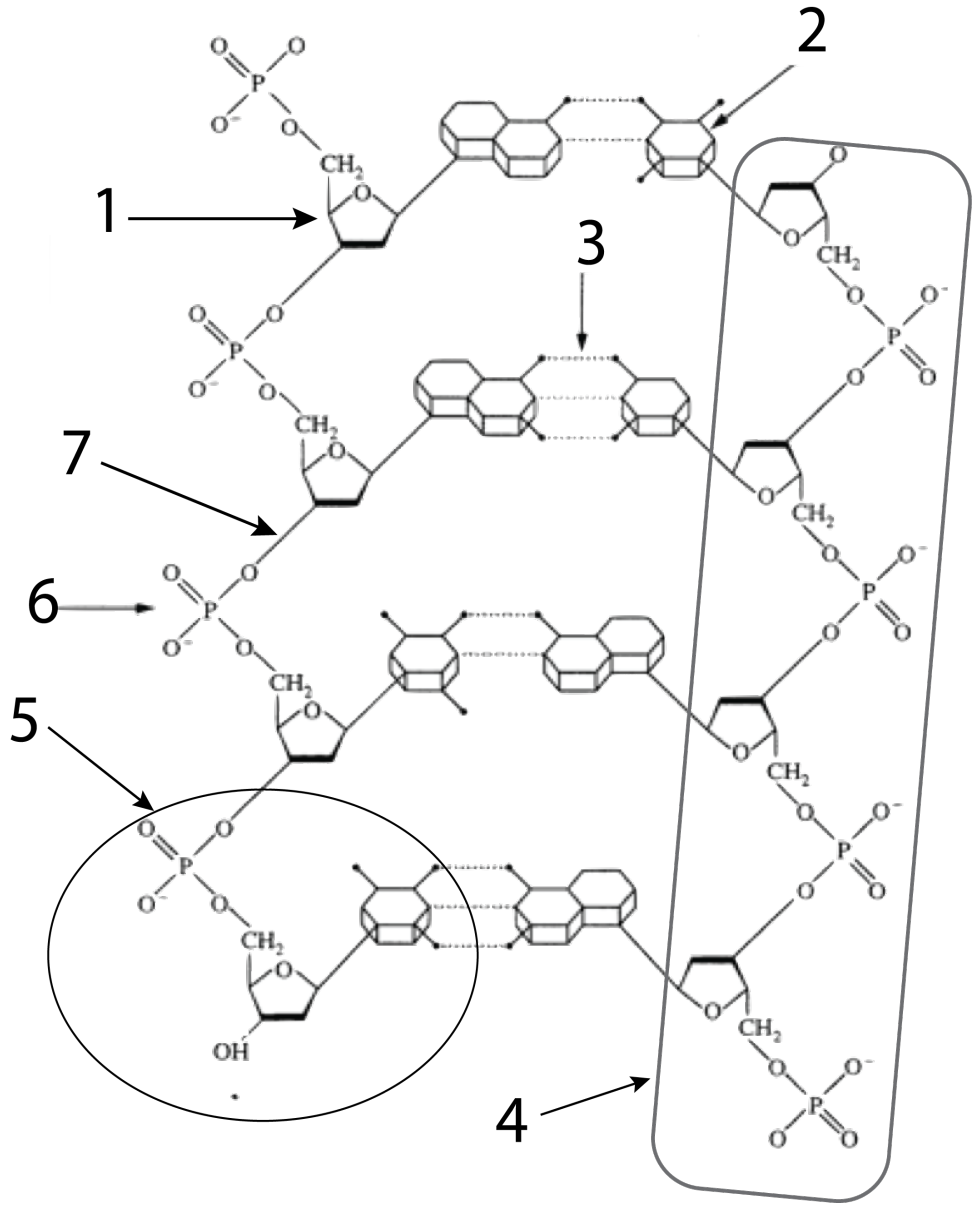



# Study your notes…

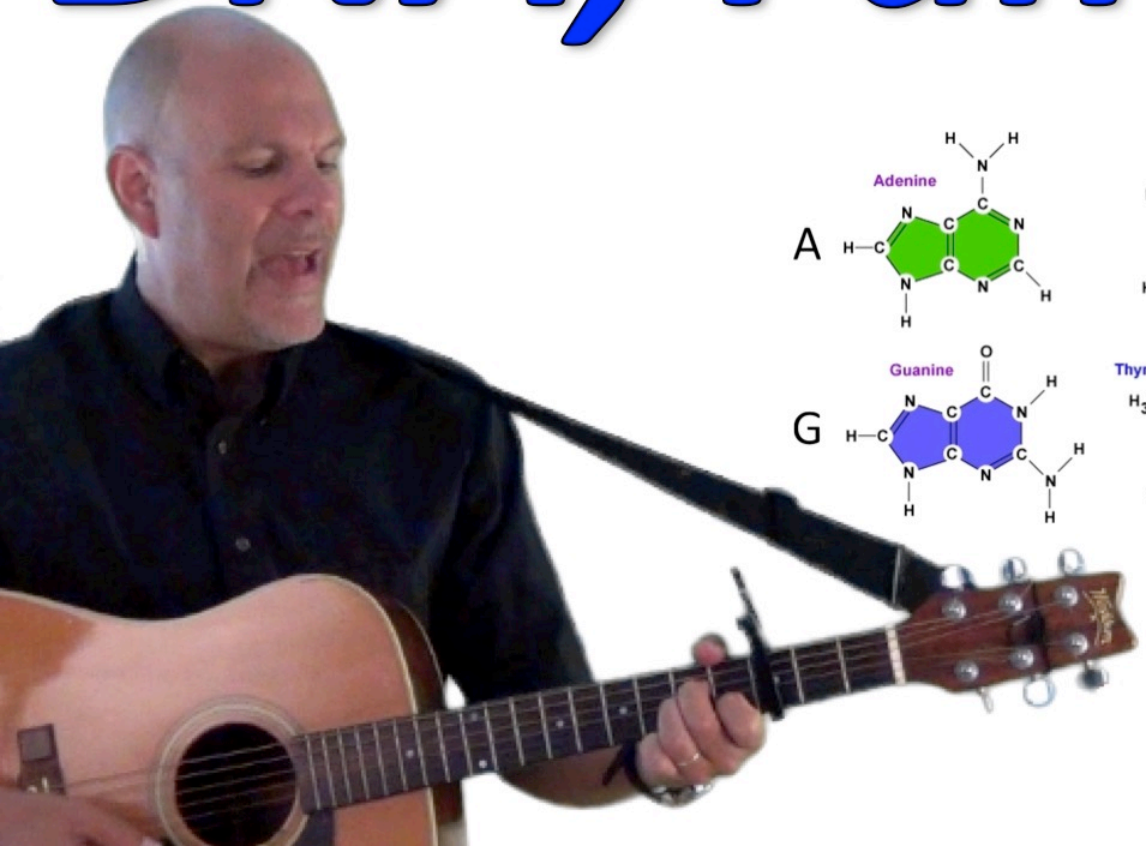
# Checking Understanding



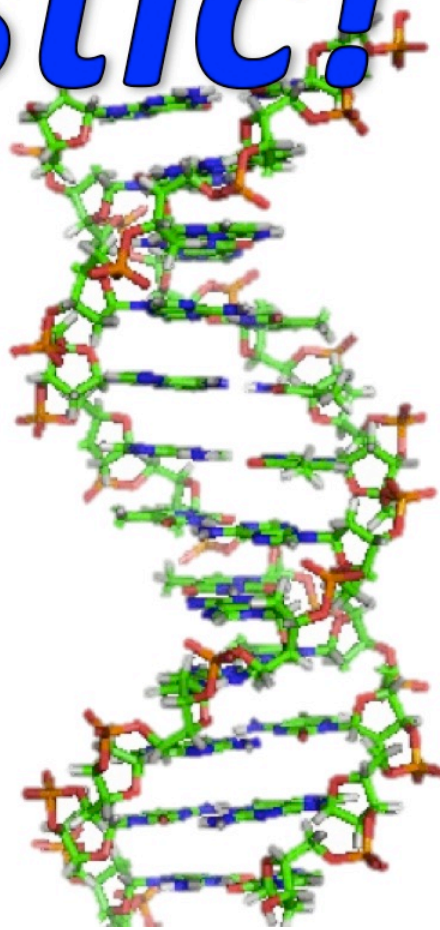
# Checking Understanding



# DNA, Fantastic!

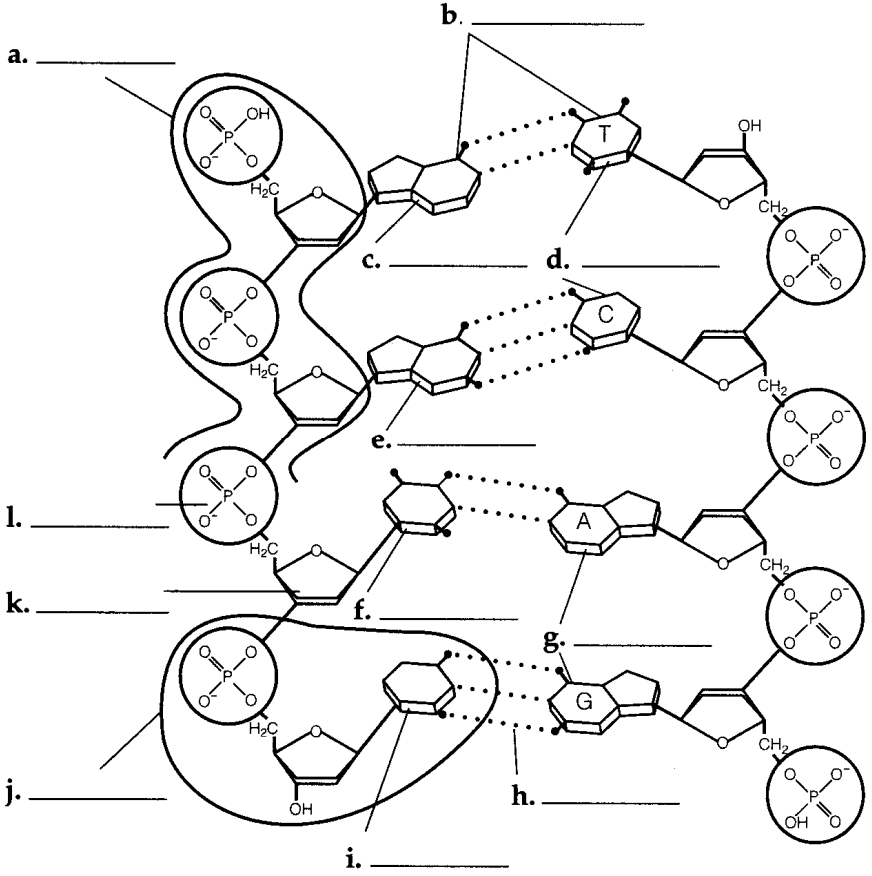


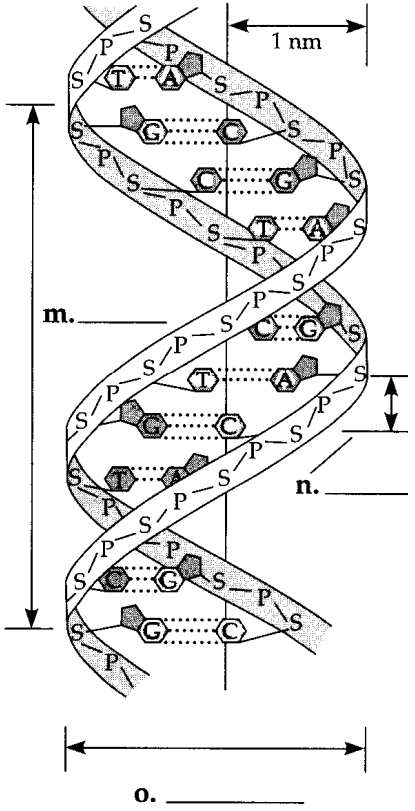


In your notes, identify parts "a" through "o" from your diagram with the terms at left. Then answer the questions and do the crossword puzzle!

**.34 nm**
**2 nm**
**3.4 nm**
**A single nucleotide**
**Adenine**
**Base pair**
**Cytosine**
**Deoxyribose**
**Guanine**
**Hydrogen bond**
**Phosphate**
**Purine bases**
**Pyrimidine bases**
**Sugar phosphate backbone**
**Thymine**

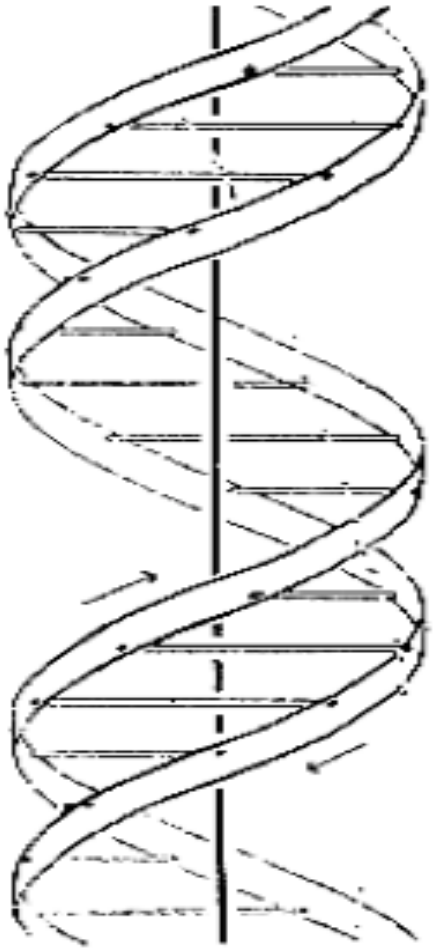


# And the answers are…

a) Sugar phosphate backbone
b) Base pair
c) Adenine
d) Pyrimidine bases (one nitrogen ring)
e) Guanine
f) Thymine
g) Purine bases (two nitrogen rings). Notice that A:T and C:G take up the same amount of space, fitting neatly inside the helix.
h) Hydrogen bond (3 between C and G; 2 between A and T).
i) Cytosine
j) A single nucleotide
k) Deoxyribose
l) Phosphate
m) 3.4 nm (10 bp: distance it takes for the helix to make one complete turn)
n) .34 nm (distance between nucleotides)
o) 2 nm: diameter of the double helix (which was determined by Rosalind Franklin's X-ray photographs)

# DNA Replication



....It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material...

Watson and Crick,

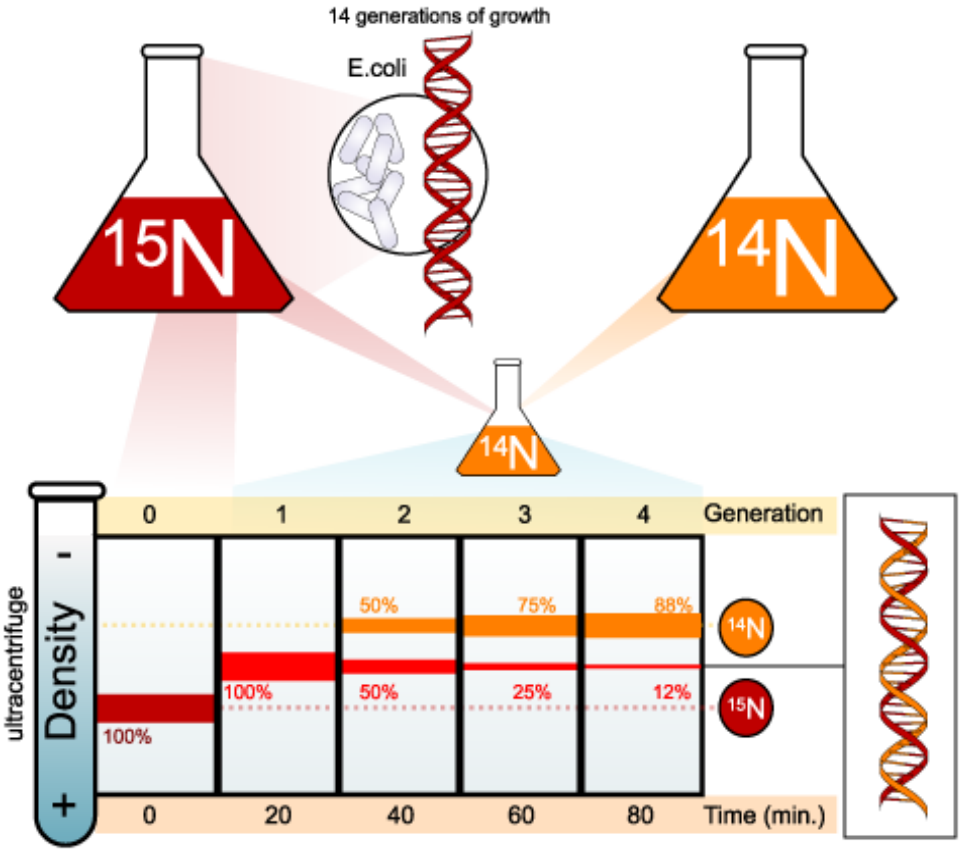
Nature, Vol. 171, 25 April 1953,

**MOLECULAR STRUCTURE OF NUCLEIC ACIDS**

# Meselson-Stahl Experiment

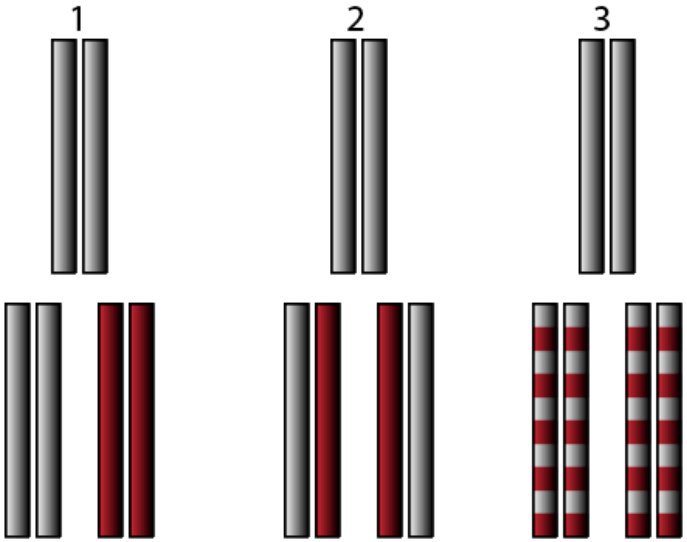
The "most beautiful experiment in biology"

Pulse-chase is a two-phase technique used to examine cellular processes that take place over a period of time. During the **pulse** phase of the experiment, cells are exposed to a labeled compound. The labeled compound is incorporated into the molecule or pathway being studied. In the **chase** phase, an unlabeled form replaces the labeled compound. The reaction is monitored to see how long it takes the labeled form of the compound to be replaced by the unlabeled form.

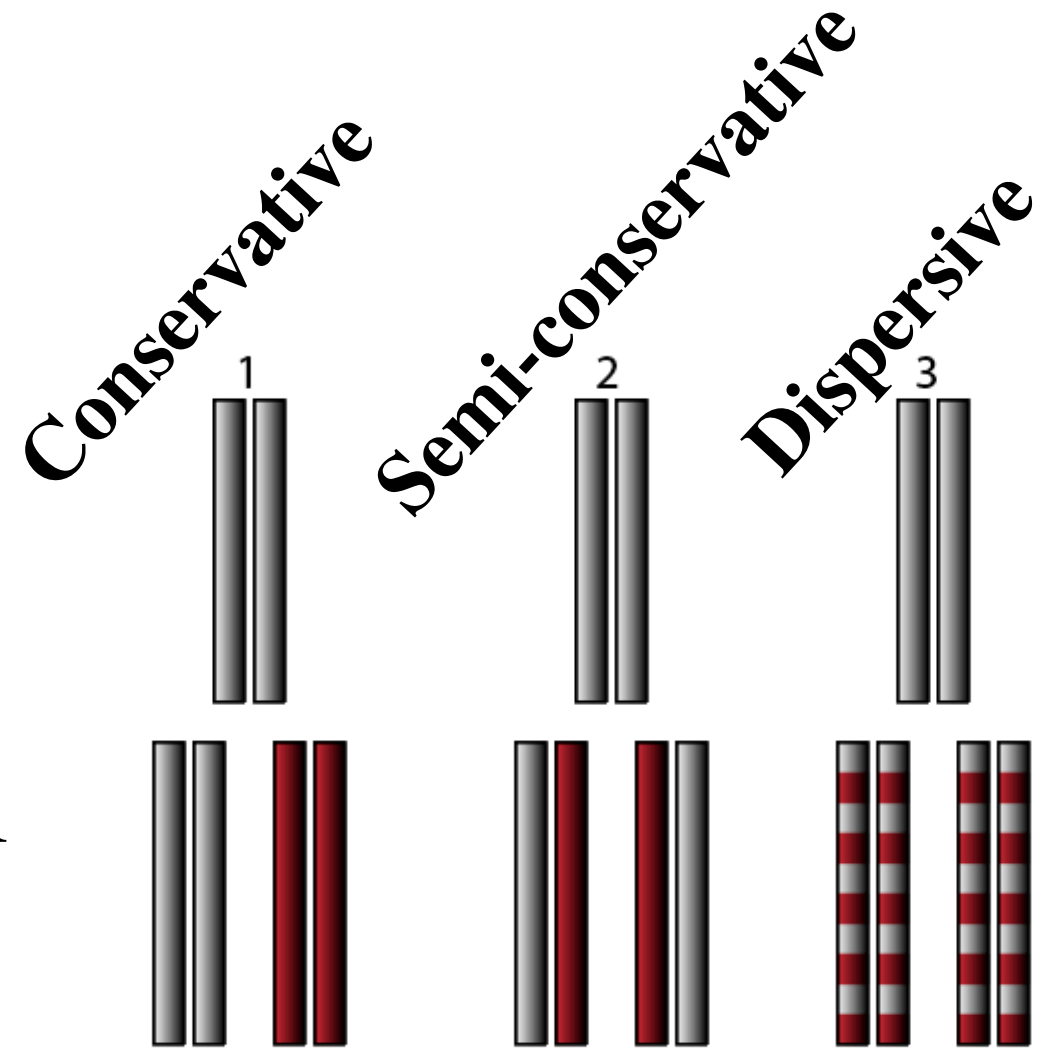


# Models of Replication

- 1, 2, and 3 are the original double stranded DNA
- The new DNA is in red
- In words, describe how these three models are different.

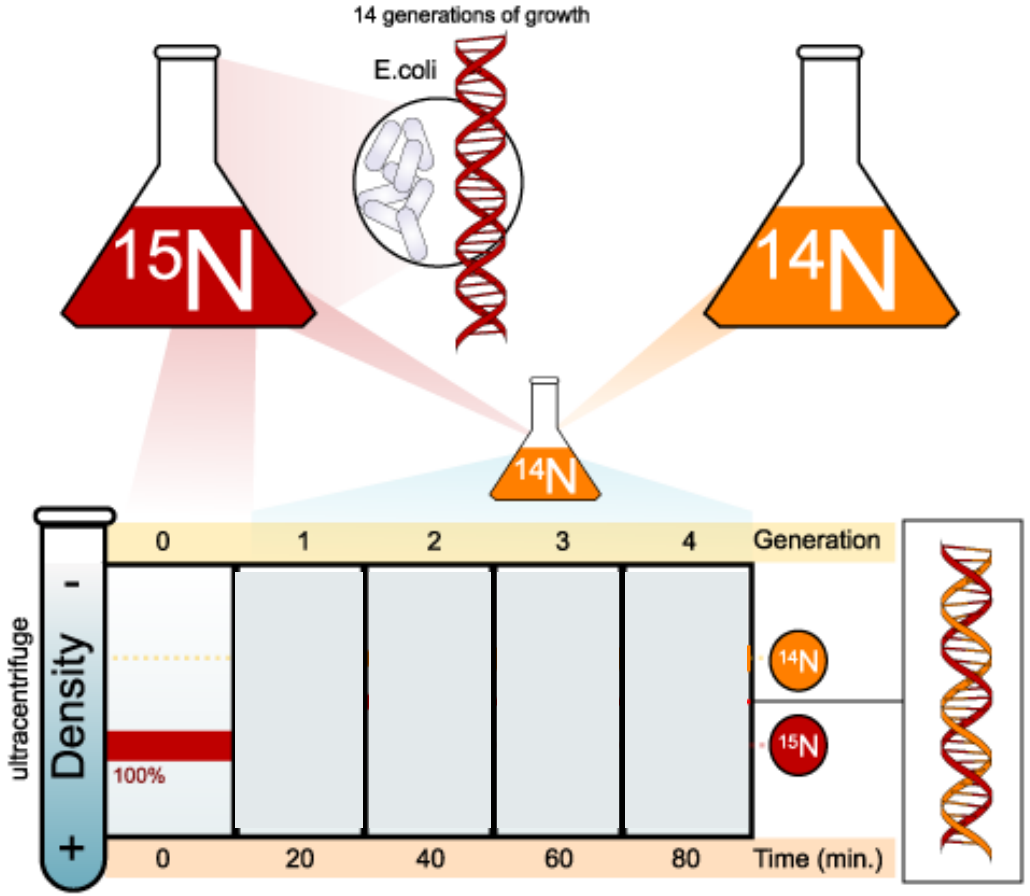


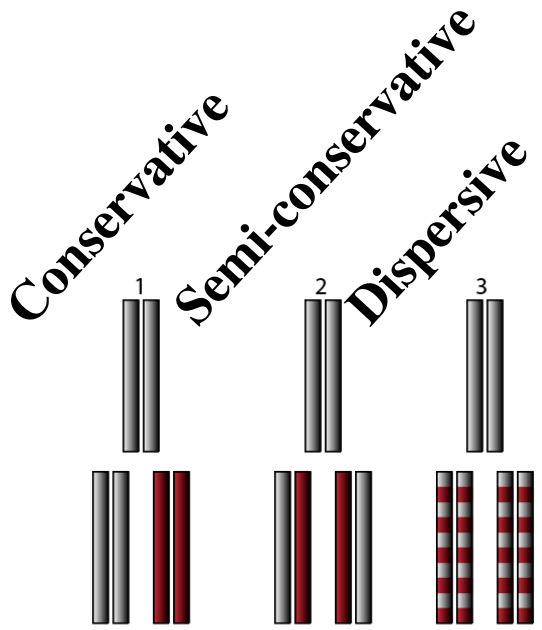
# Models of Replication



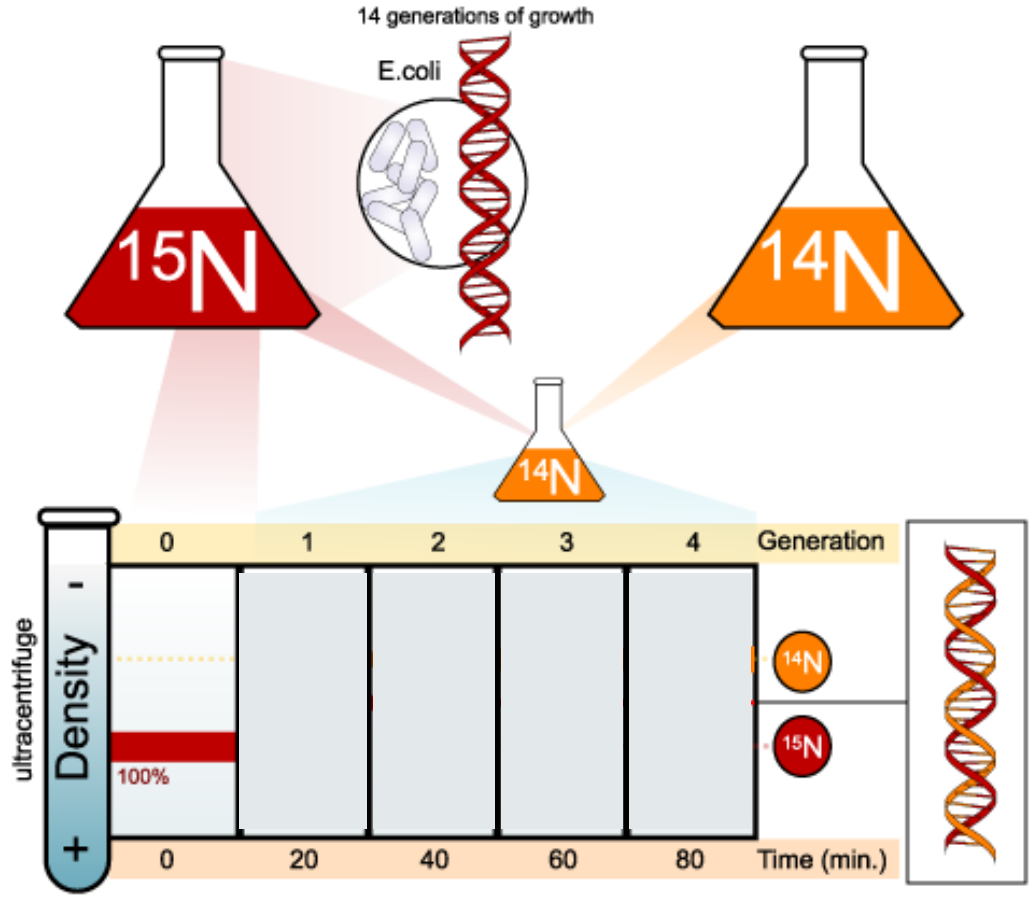
# Meselson Stahl Experiment (method)

1. Grow *E. coli* in a medium with heavy isotope of nitrogen ($^{15}N$)
2. Transfer $^{15}N$ bacteria to a medium with normal nitrogen ($^{14}N$)
3. As bacteria reproduce, they can only use $^{14}N$
4. Every generation, test the density of the bacterial DNA.






# *Predict!*

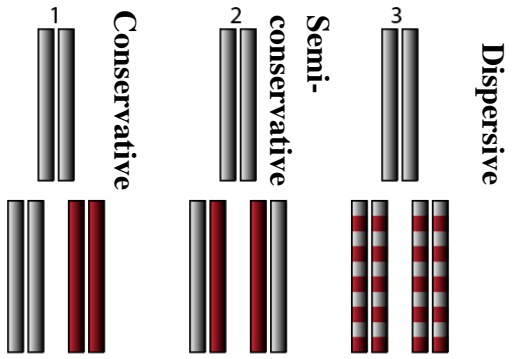


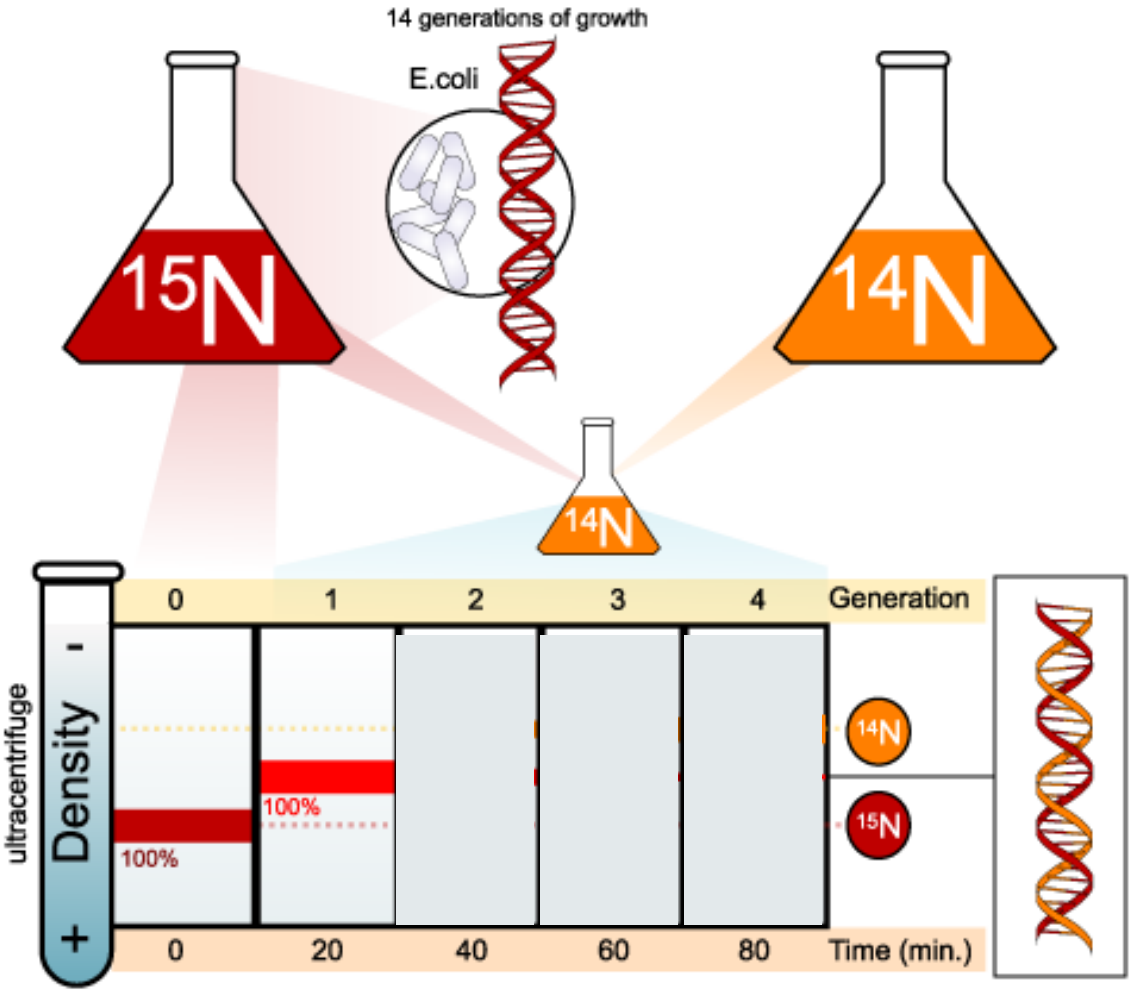

- *Density in generation 0 is 100% $^{15}N$.*
- *What will be the density in generation 1? Generation 2?*

# Meselson Stahl Results (1)

5. Generation 0: all DNA has the density of $N^{15}$.
6. Generation 1: All the DNA has density of $N^{14.5}$

What does generation 1 prove?

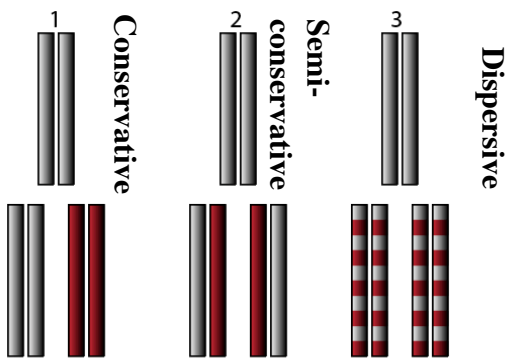


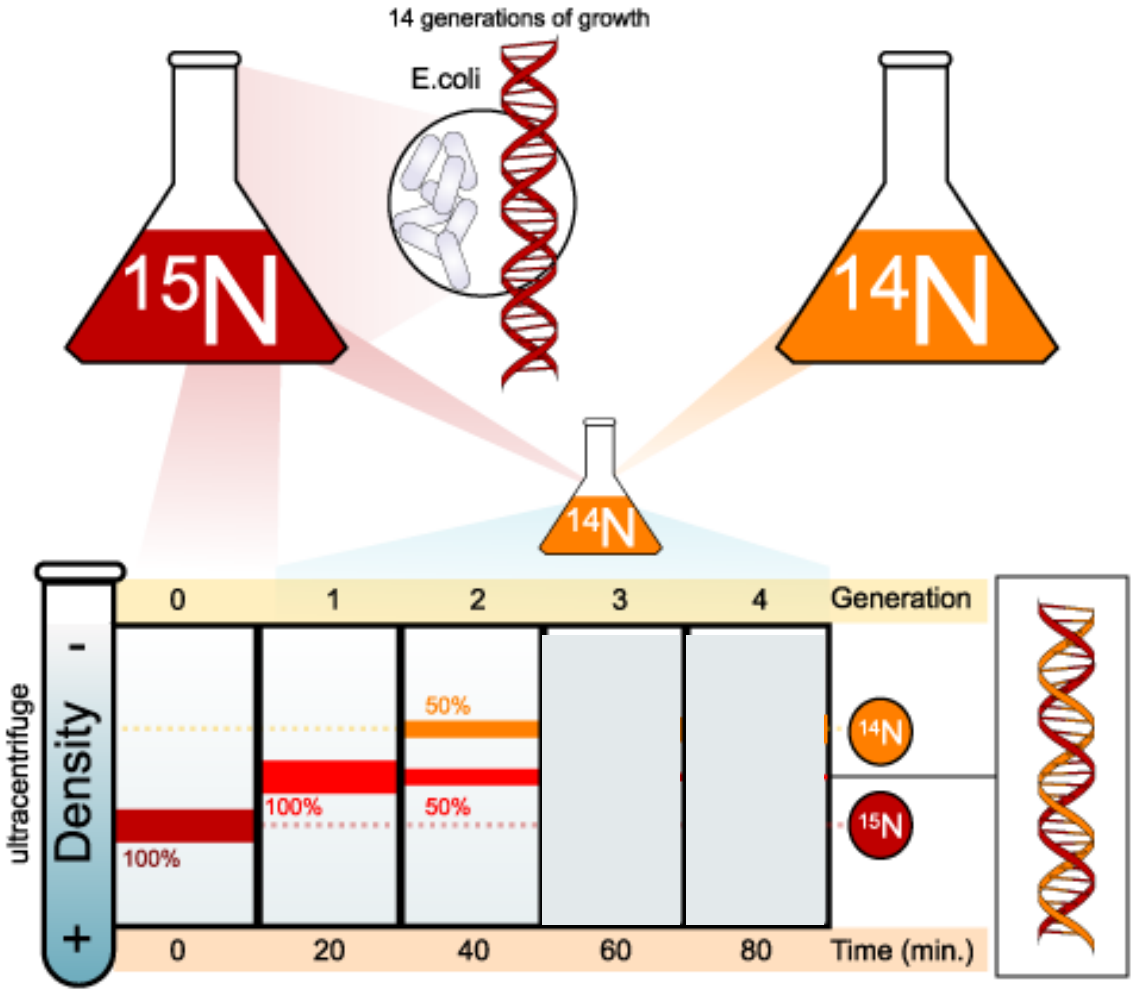



# Meselson Stahl Results (2)

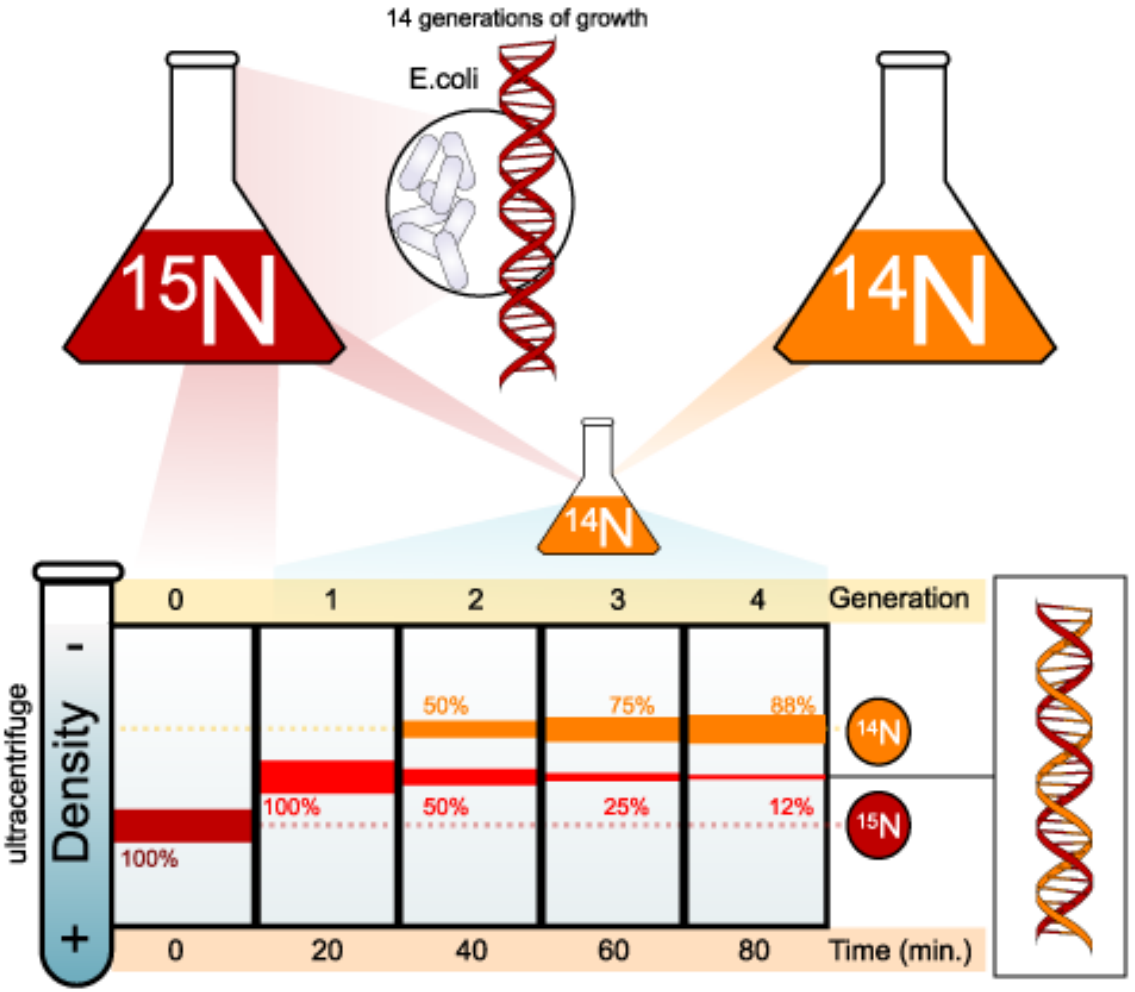
7. Generation 2:
    1. Some DNA has density of 14.5
    2. Some DNA has density of 14
8. After Generation 3: same as 2

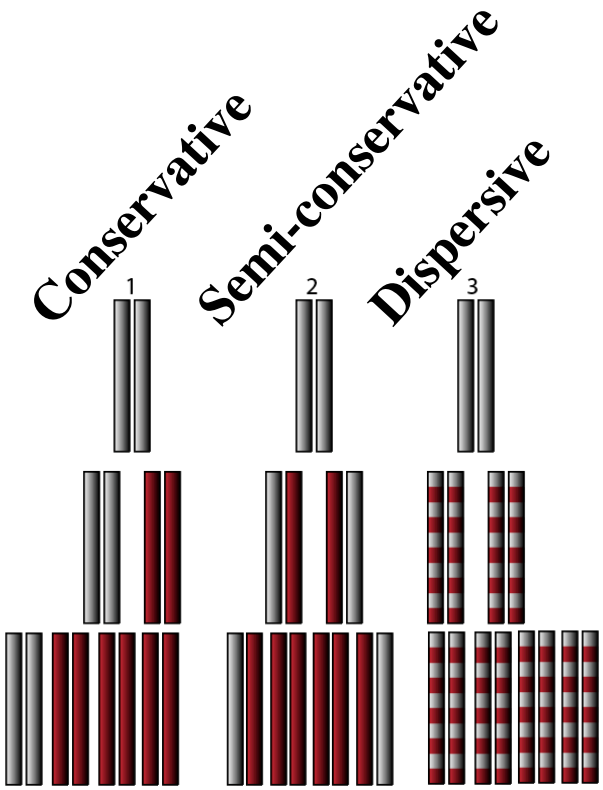
What does generation 2 prove?



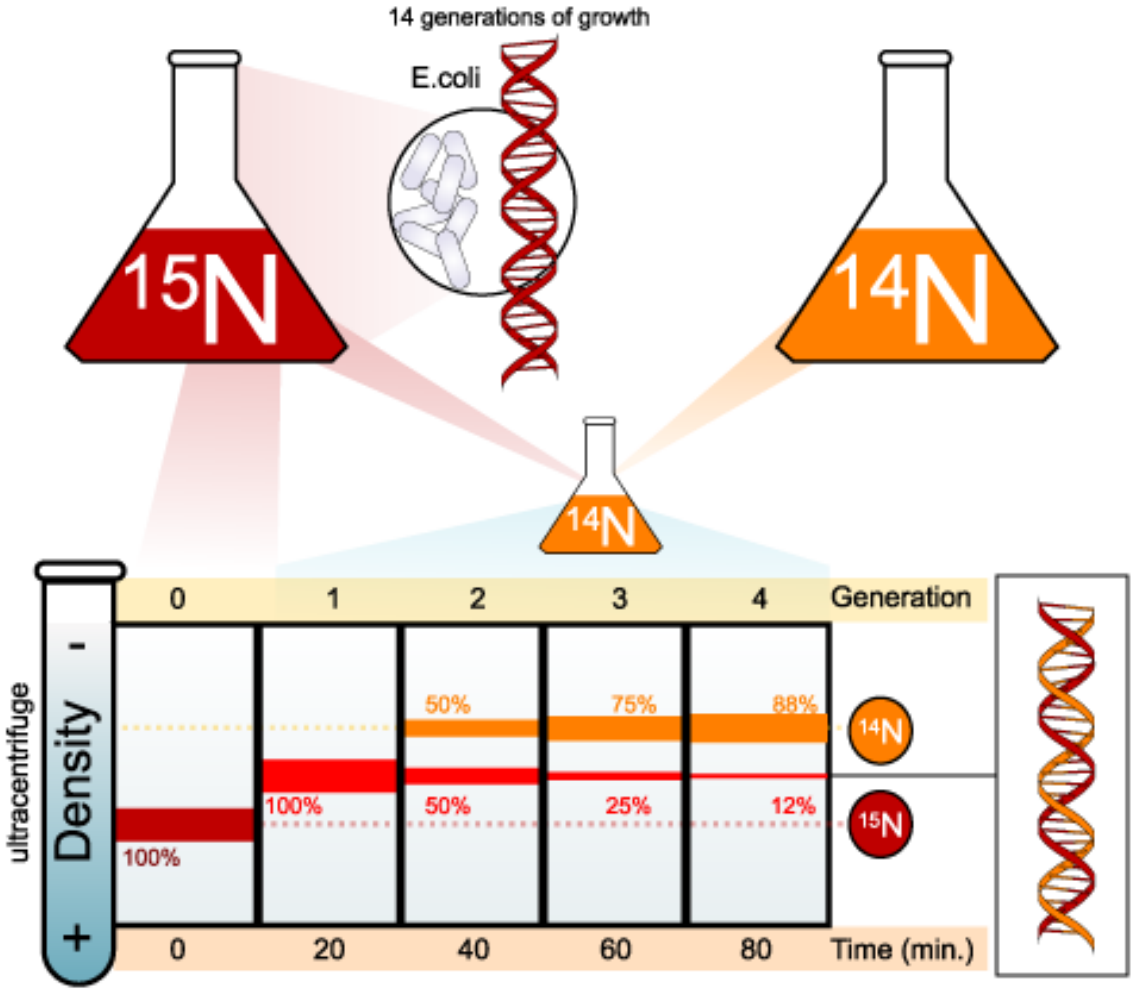



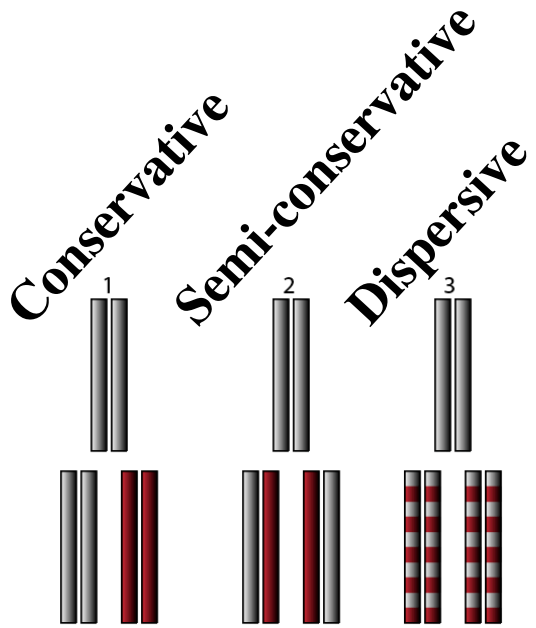
# Meselson Stahl Results



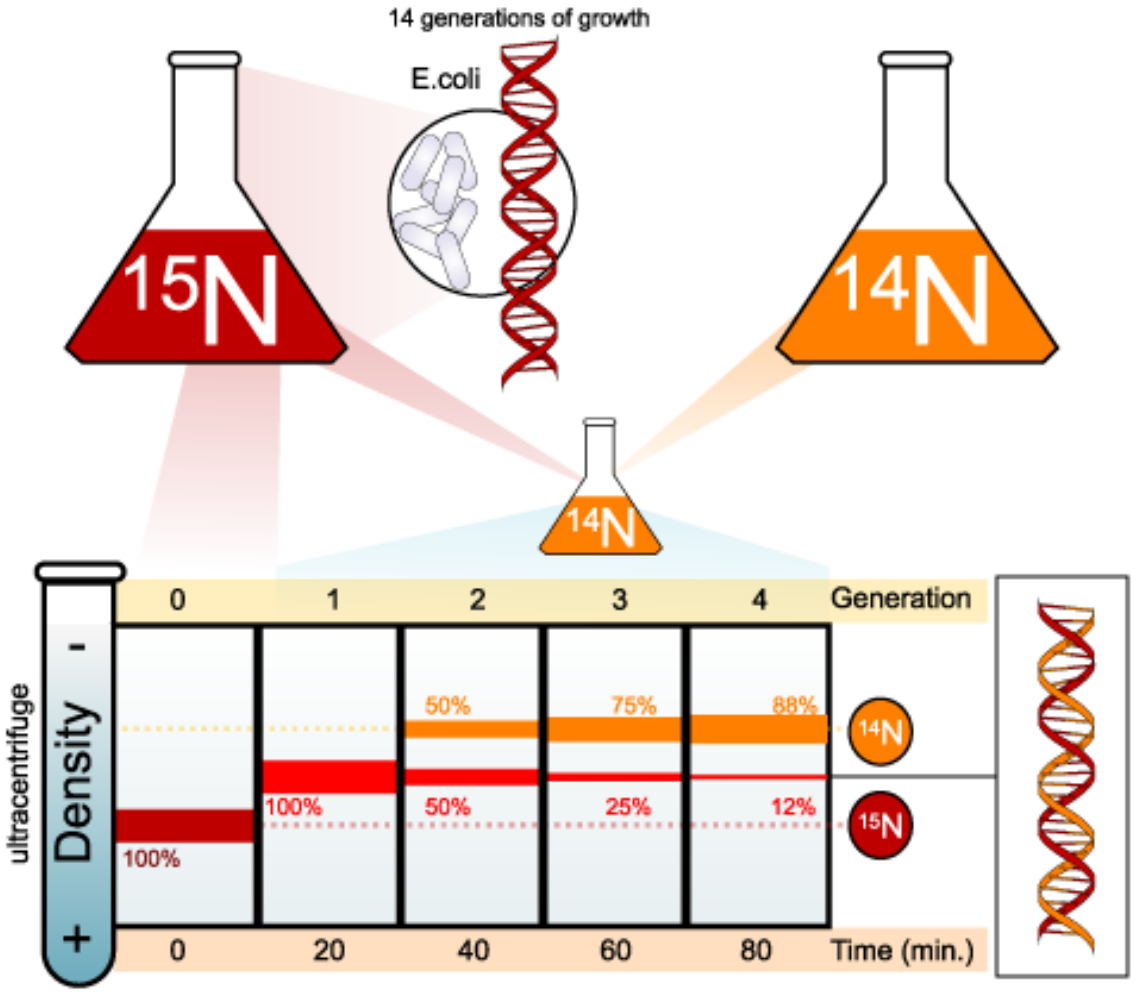



# Which Model is Correct?

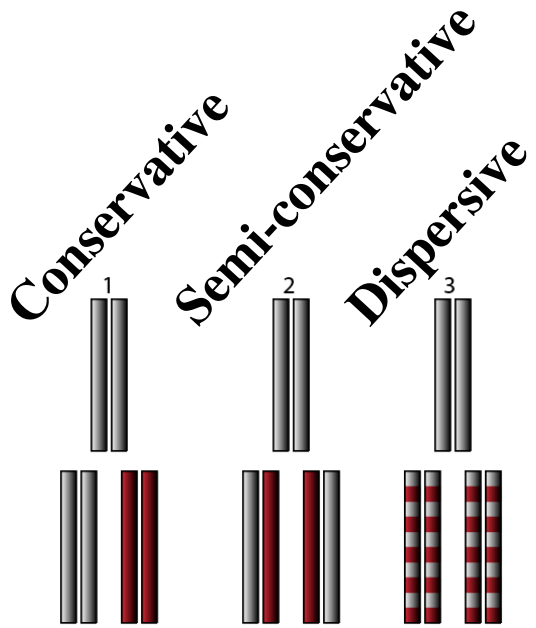




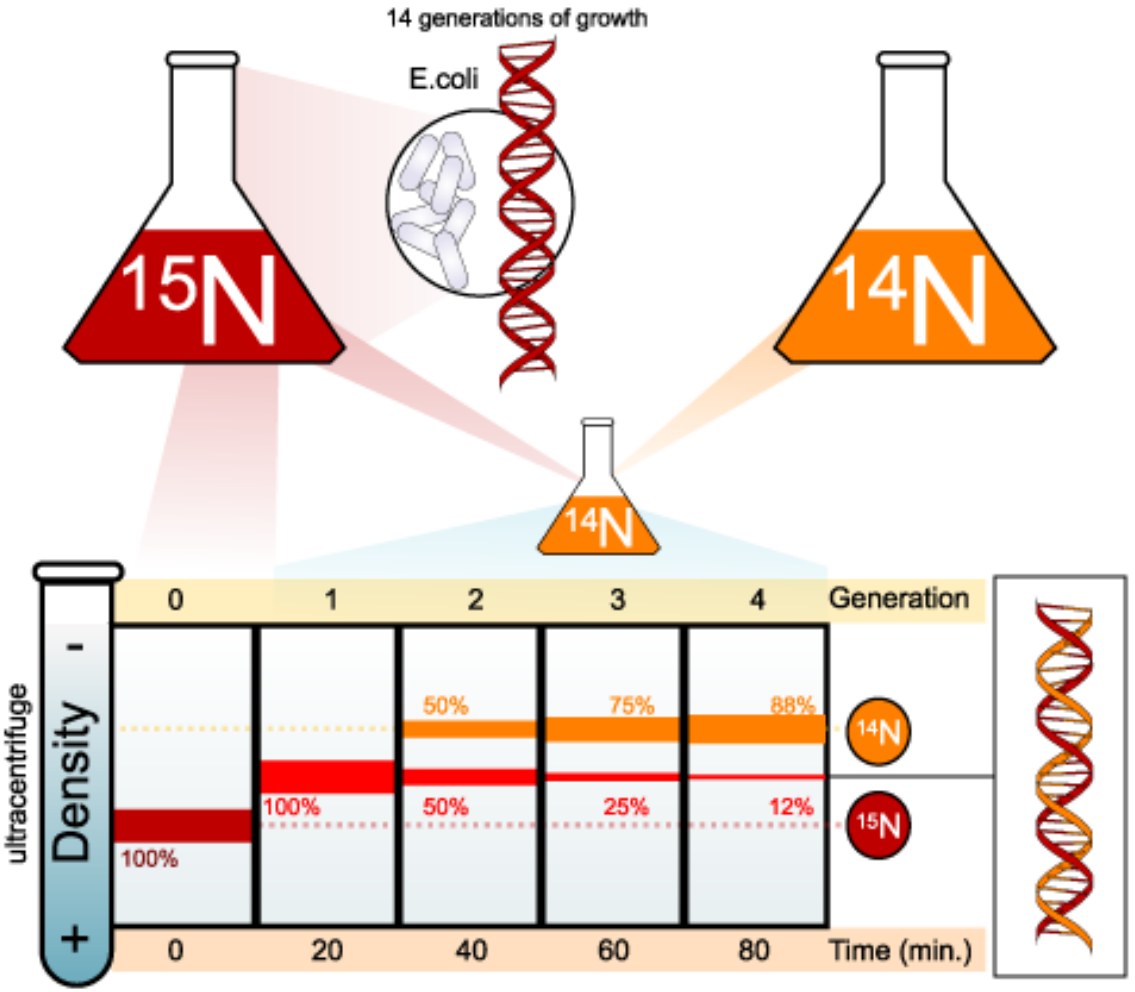

# Which Model is Correct?




- Generation 1 eliminates _____
- Generation 2 eliminates _____




# Meselson-Stahl

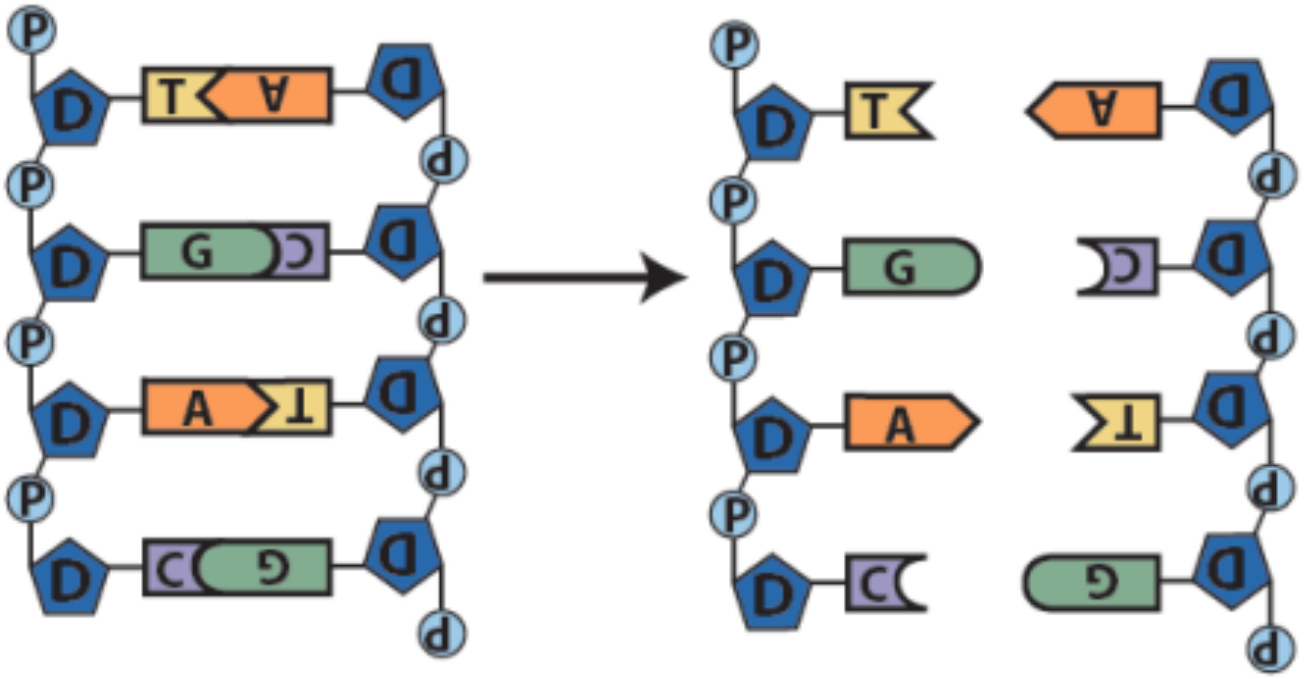



Write a brief summary of how they established semi-conservative replication as the mode of DNA replication.

# So, how is DNA actually copied

# DNA Replication: Big Picture (1)

- The strands separate

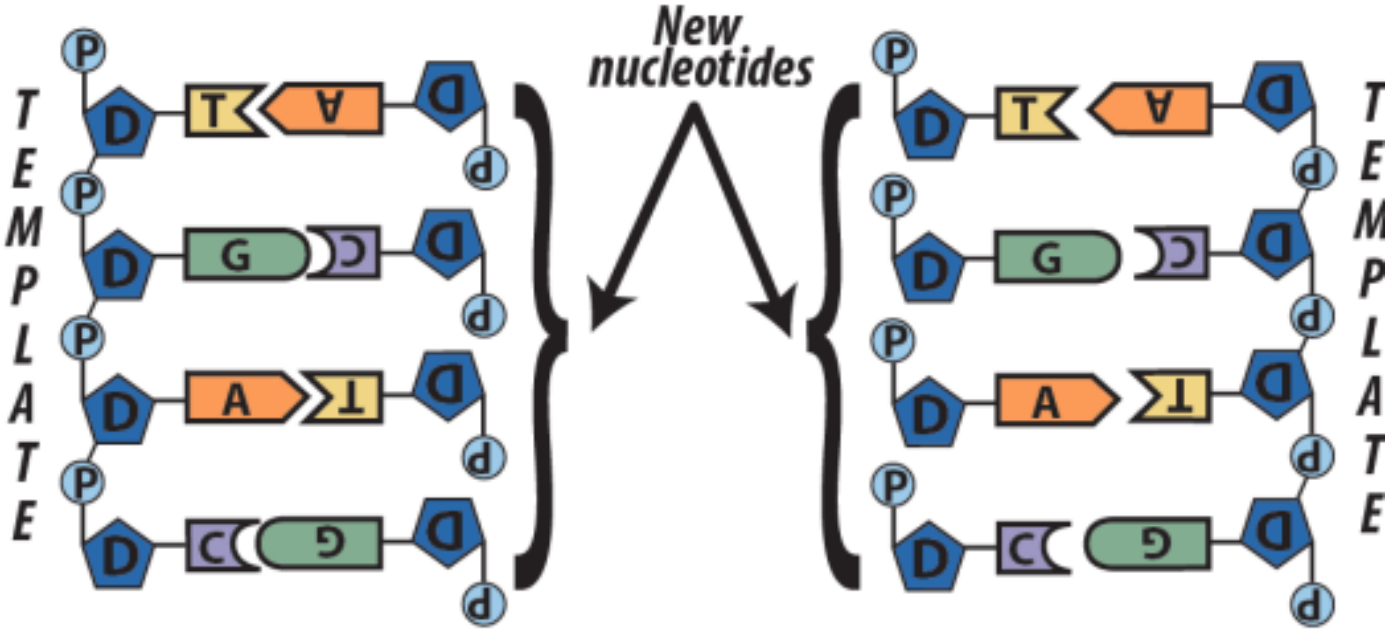


# DNA Replication: Big Picture (2)

- Each single strand now serves as a template for synthesis of a new strand.

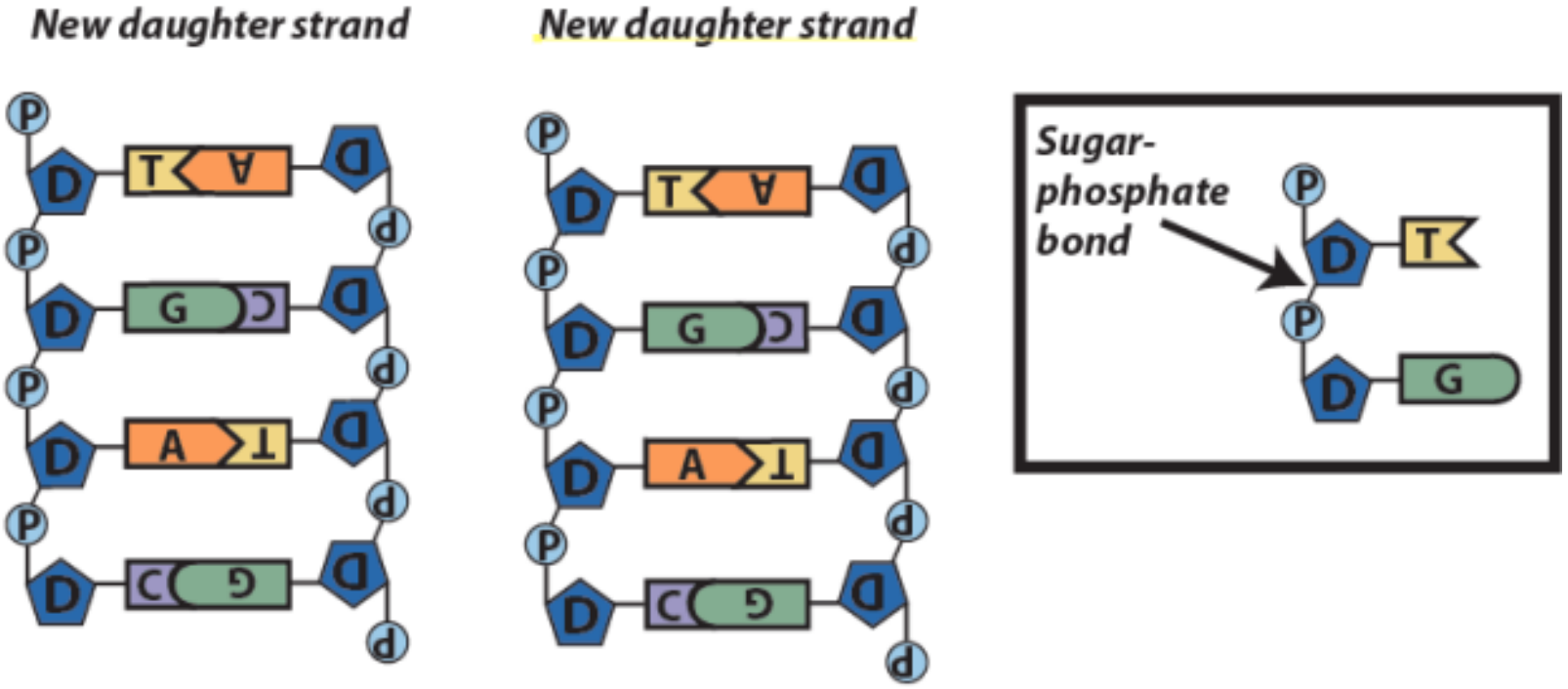
# DNA Replication: Big Picture (3)

- New complementary nucleotides bind with the parent strands

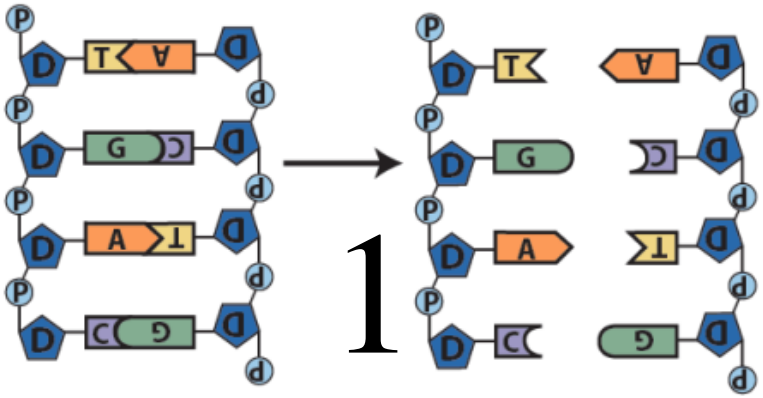


# DNA Replication: Big Picture (4)

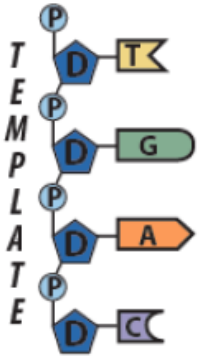
- Enzymes seal covalent bonds between the sugars and the phosphates of adjacent nucleotides.



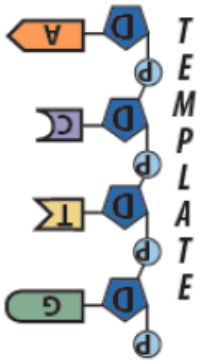
# Explain the whole process

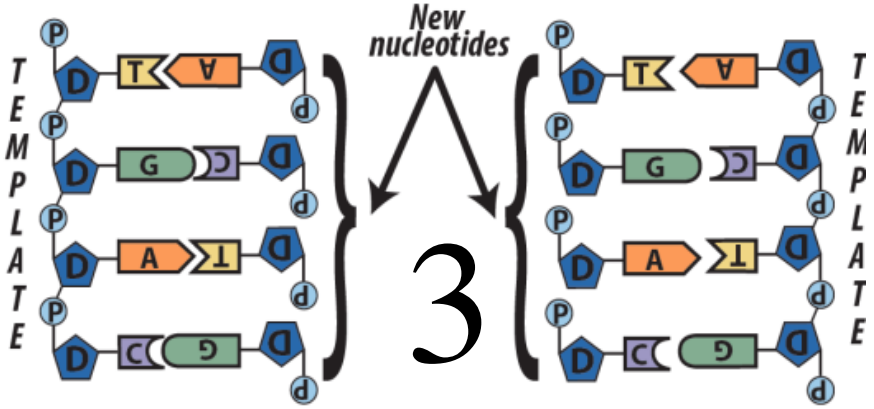


1

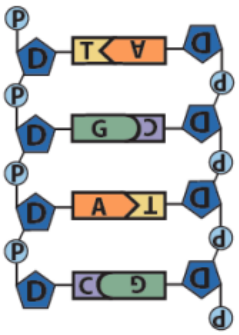


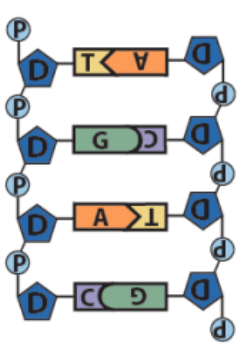

2

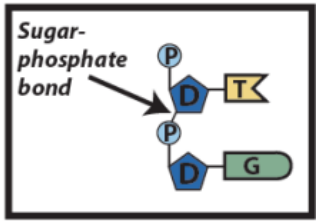



3

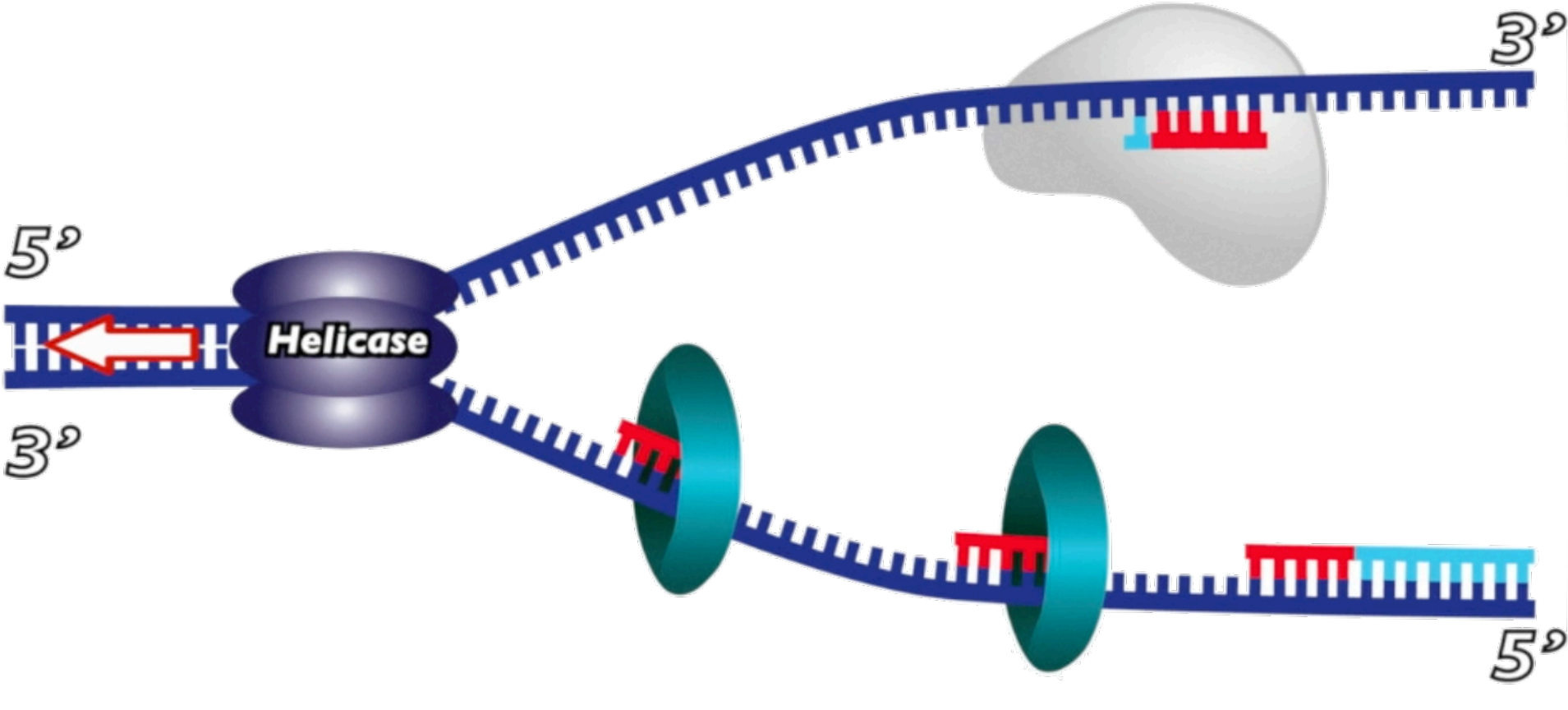


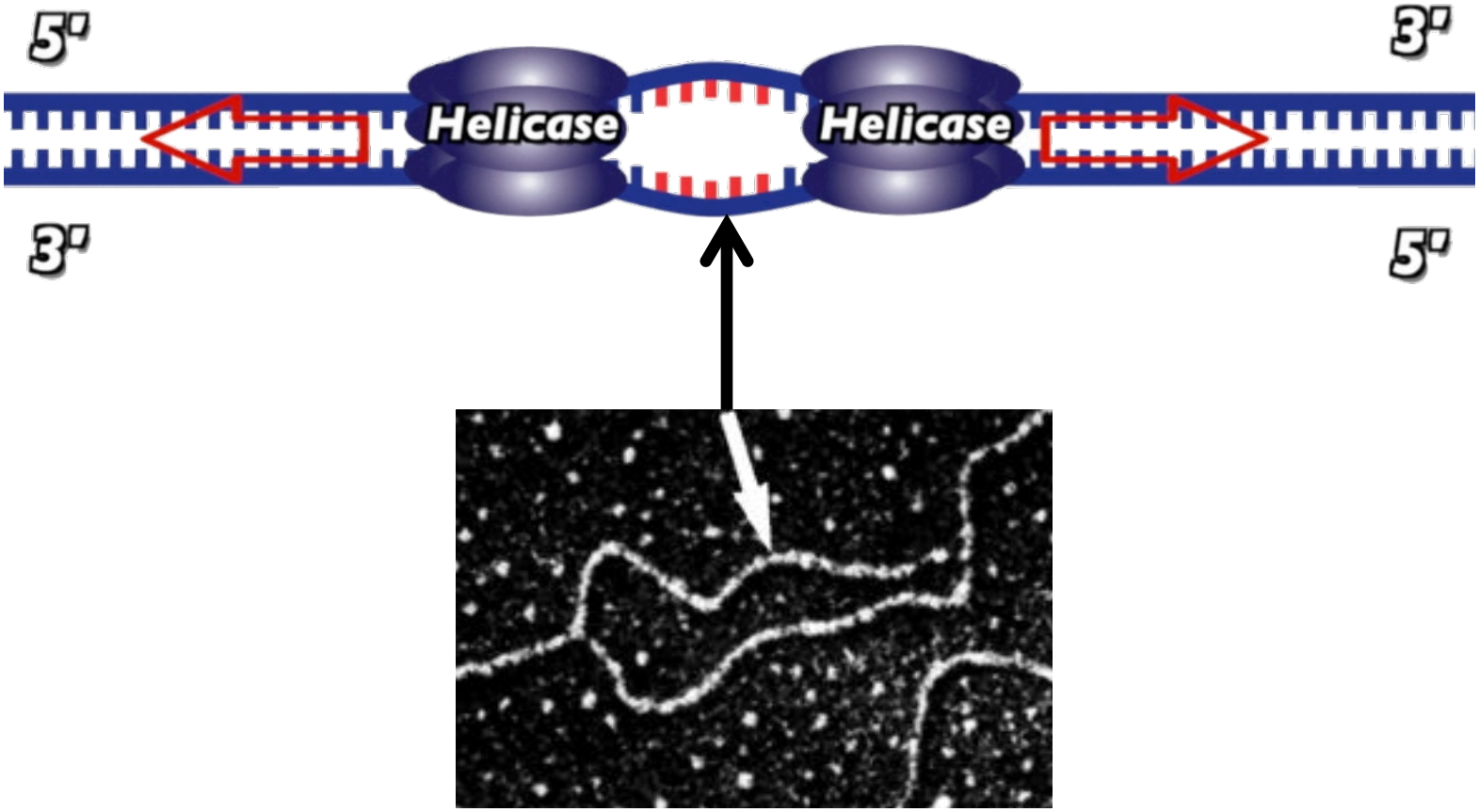




4

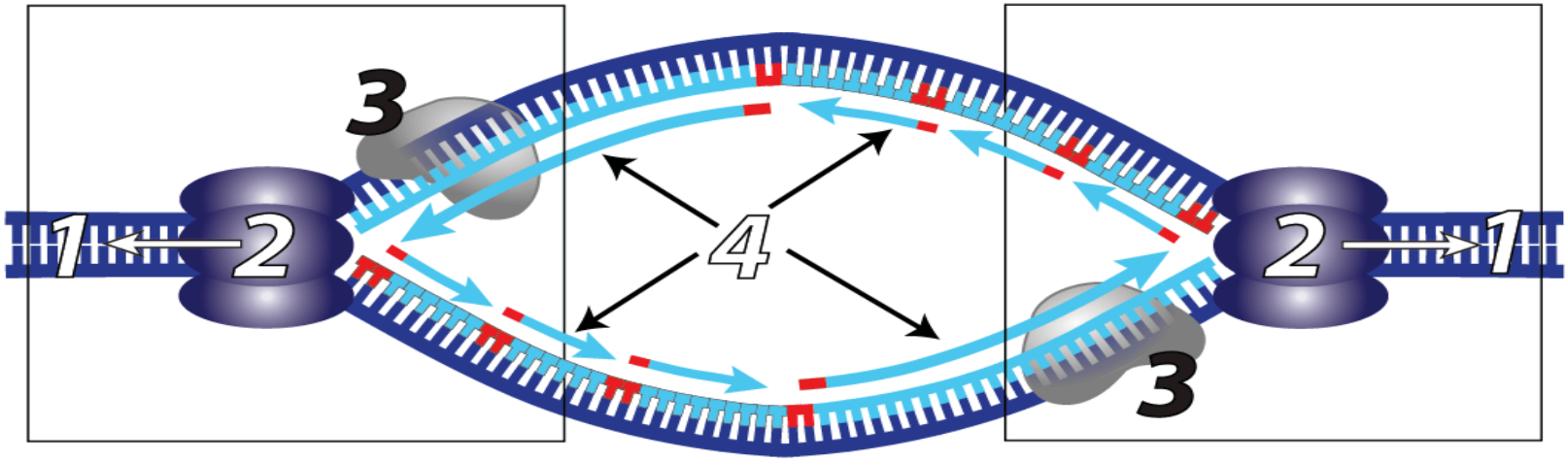
# In cells, blind, mindless, enzymes have to do this entire process by touch and feel…



# Replication begins as DNA helicase finds an *origin*, and creates a *replication bubble*

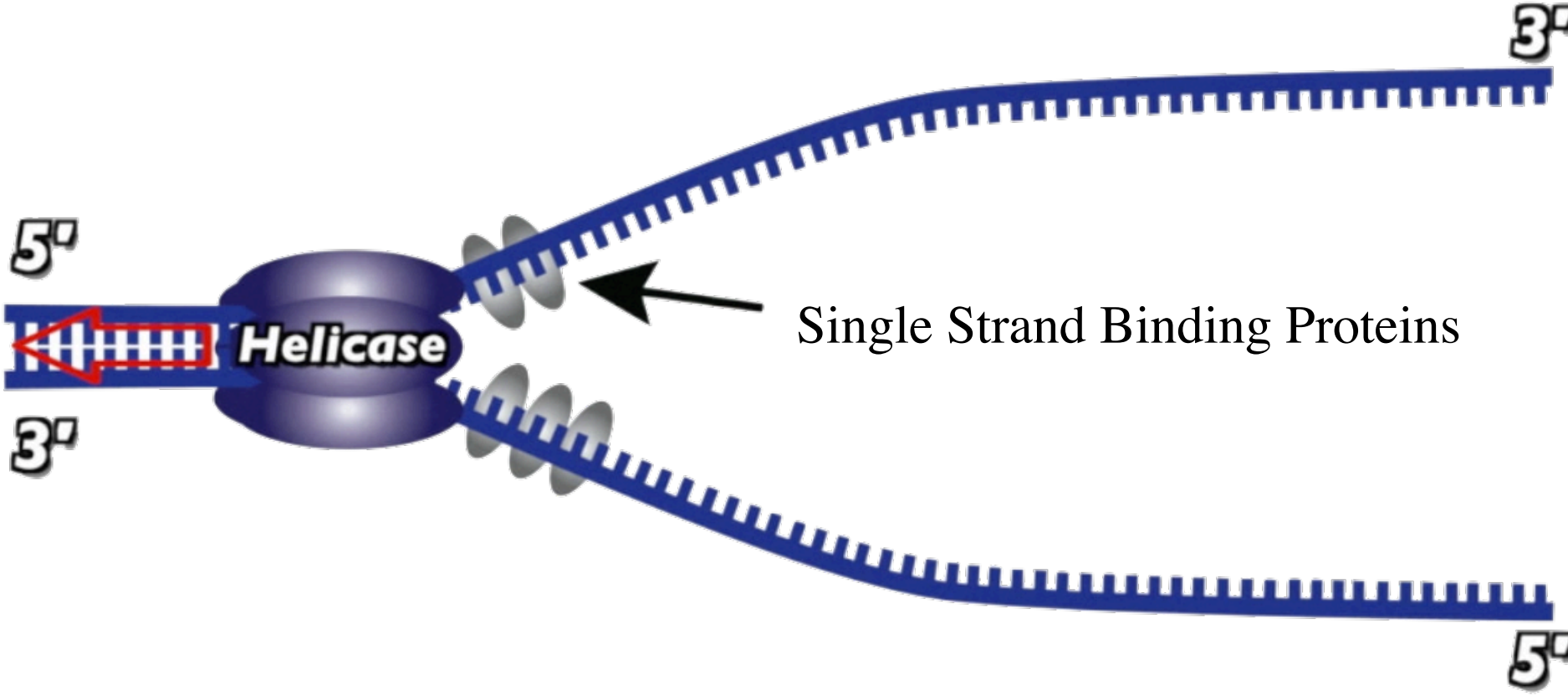


# The bubble has two *replication forks*

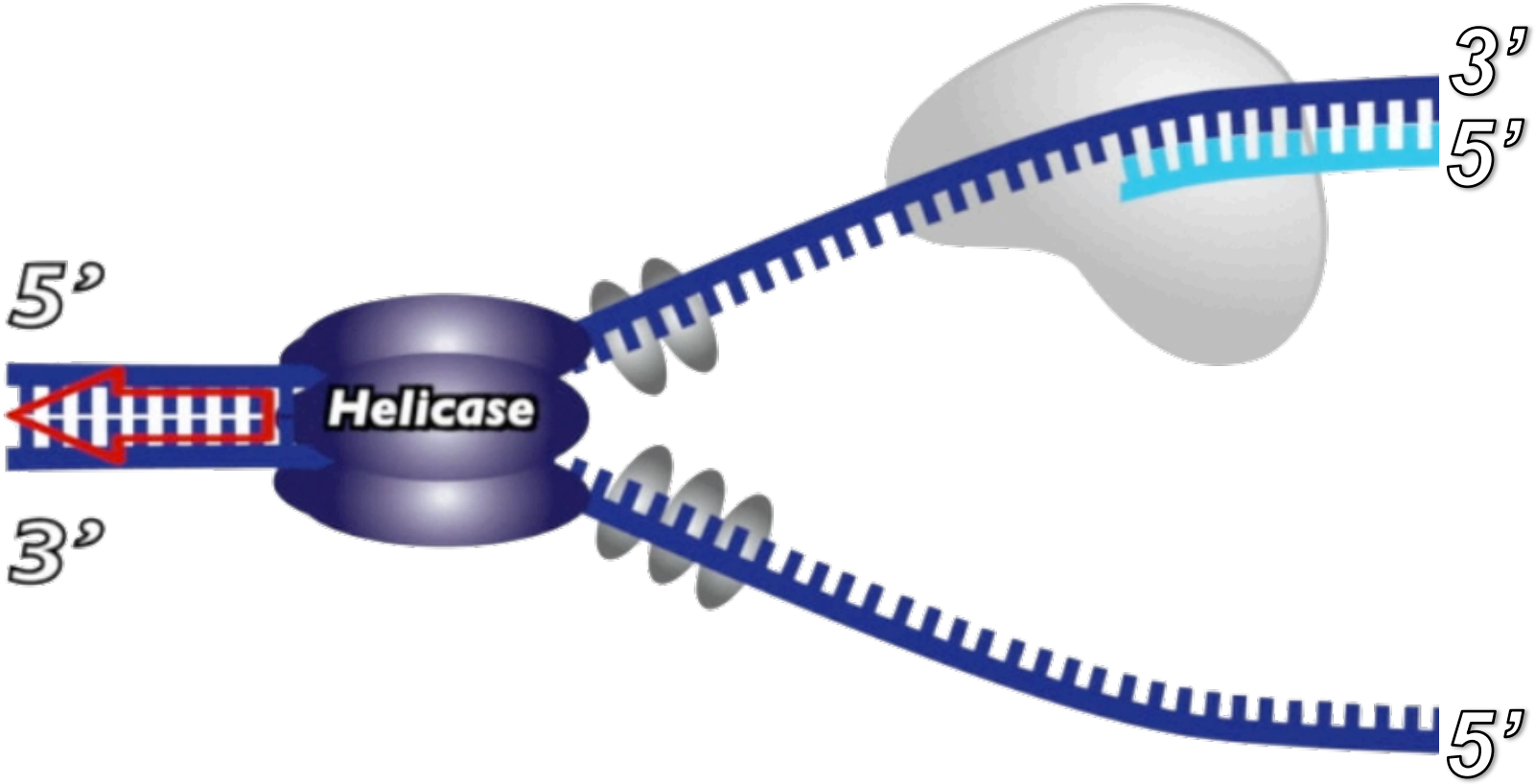



1. Original DNA
2. Helicase
3. DNA polymerase
4. New DNA

# Single Strand Binding Proteins keep DNA from rewinding

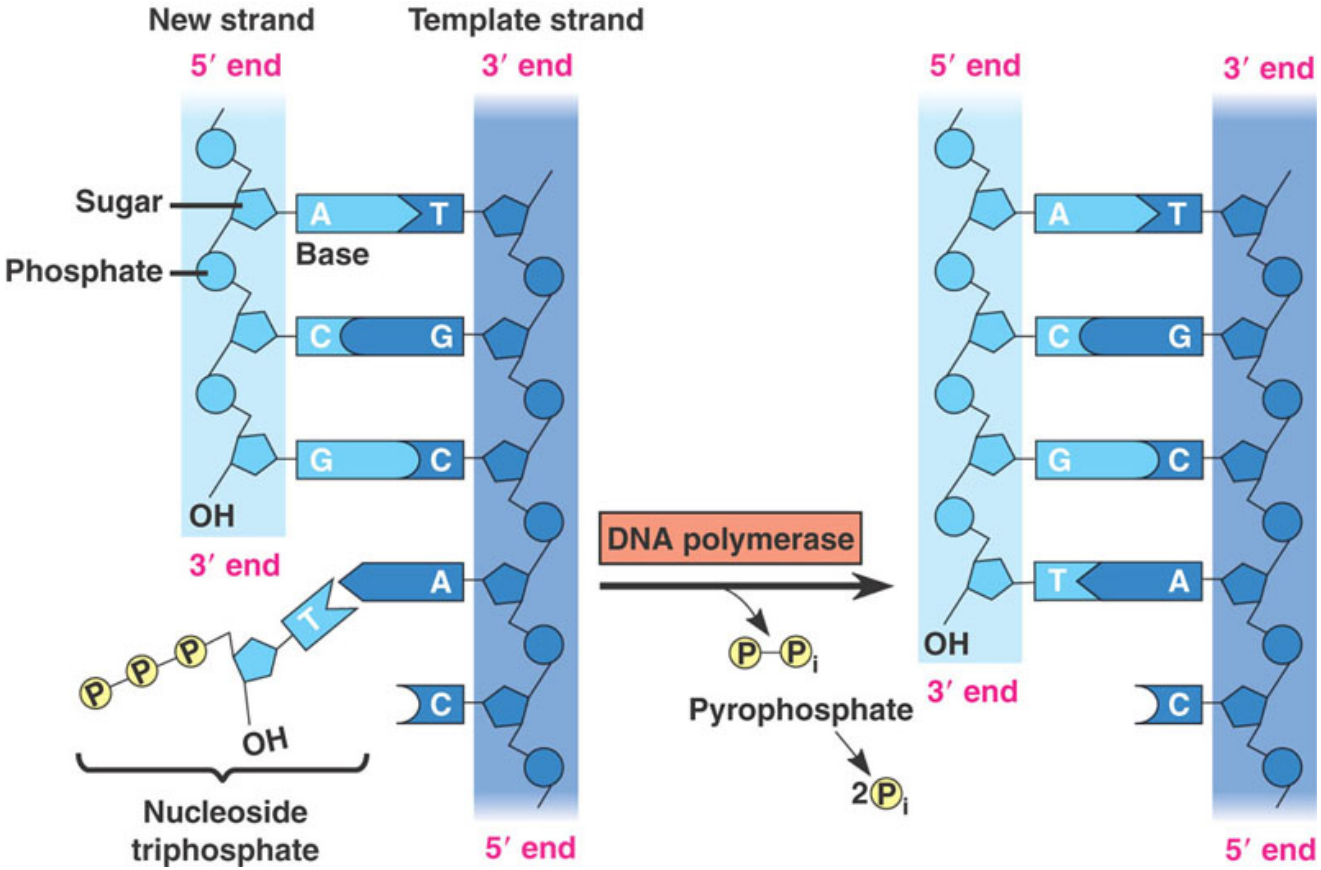


# DNA polymerase III uses the parent strand as a template and adds a new nucleotide at the 3’ end



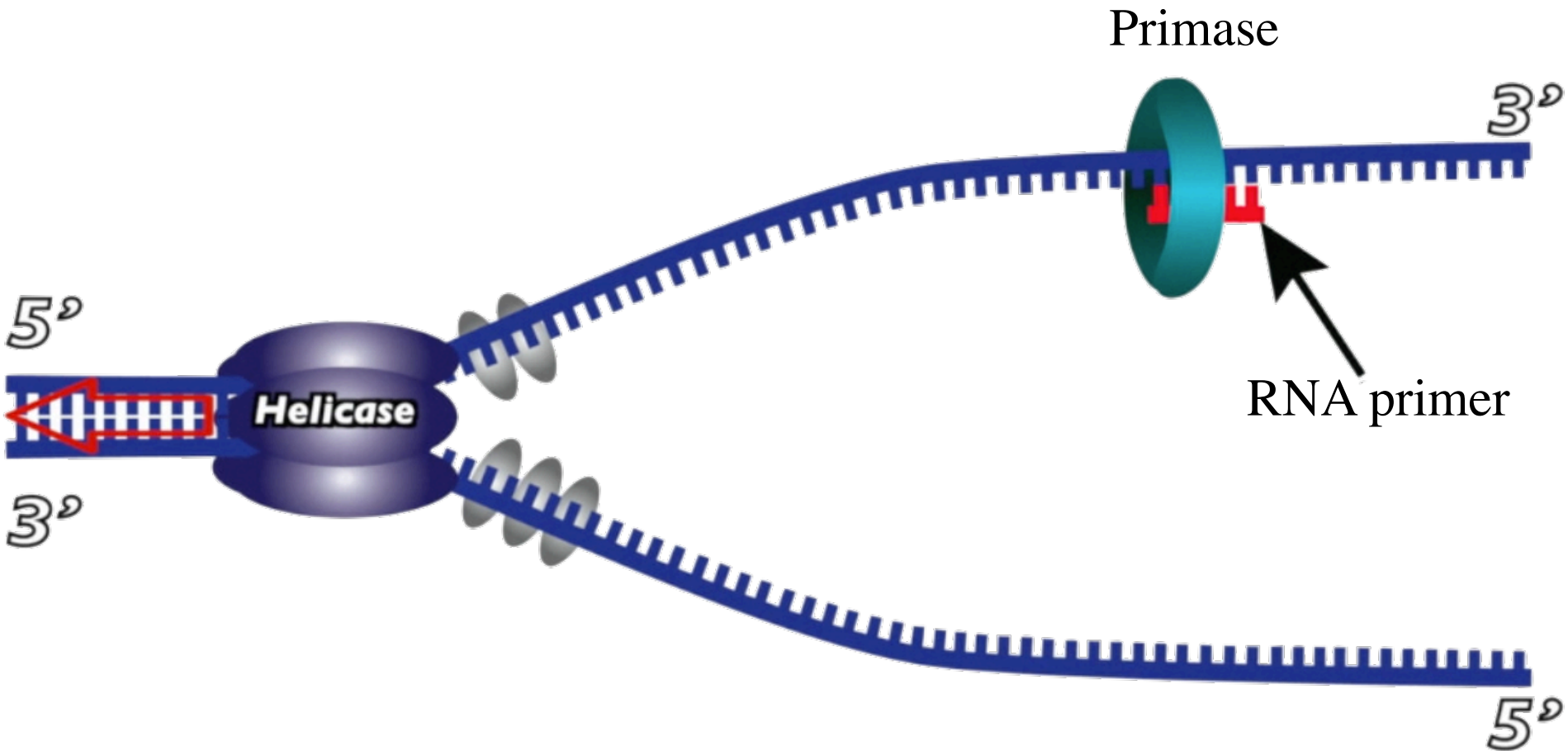
# DNA polymerase III.

- Waits for free nucleotides to H-bond with bases on the *template strand*.
- Creates sugar-phosphate bond between existing strand and new nucleotide at the 3' end.
- Energy comes from phosphate groups on nucleotides.

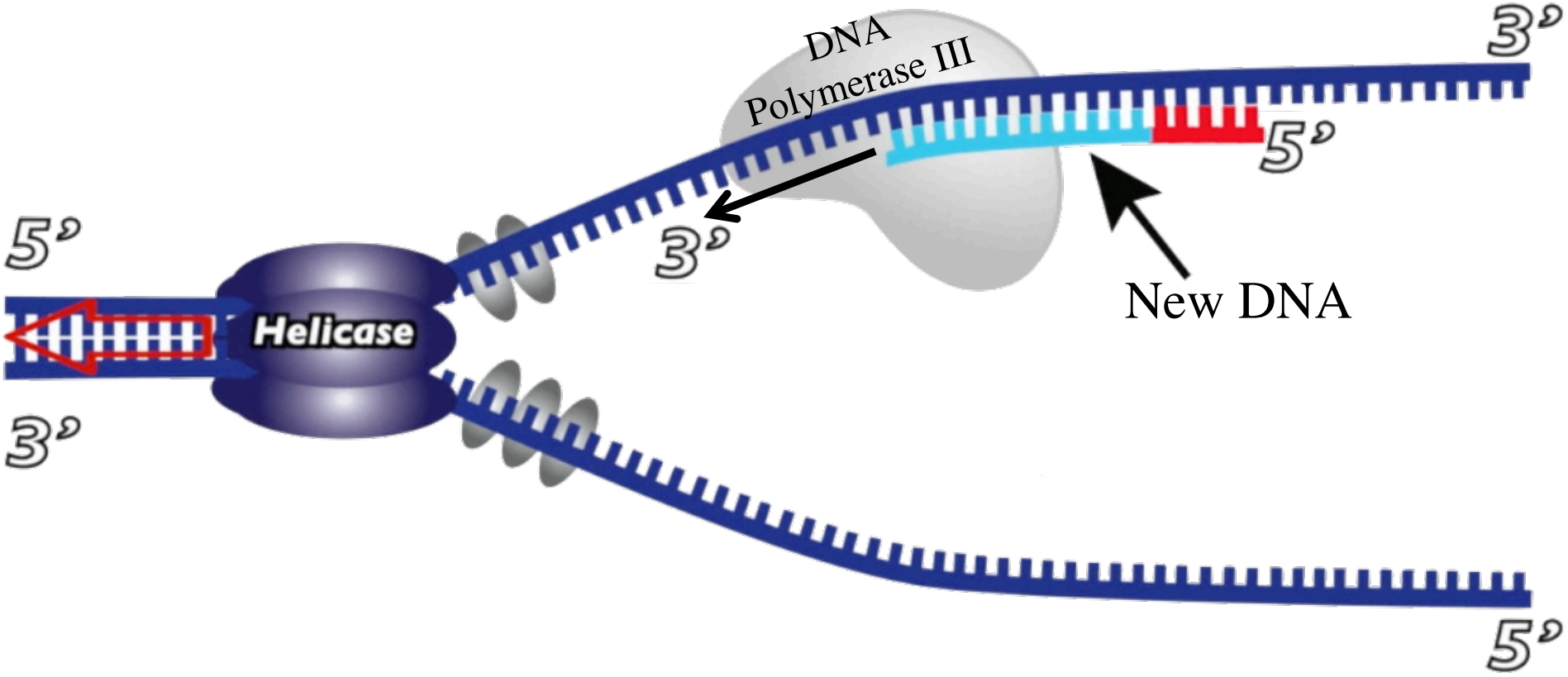


# Replication starts with *Priming*

- DNA polymerase III can only add to an existing strand.
- *Primase*
  - Starting from origin, lays down a short strand of complementary RNA
  - Works in 5’ to 3’ direction.

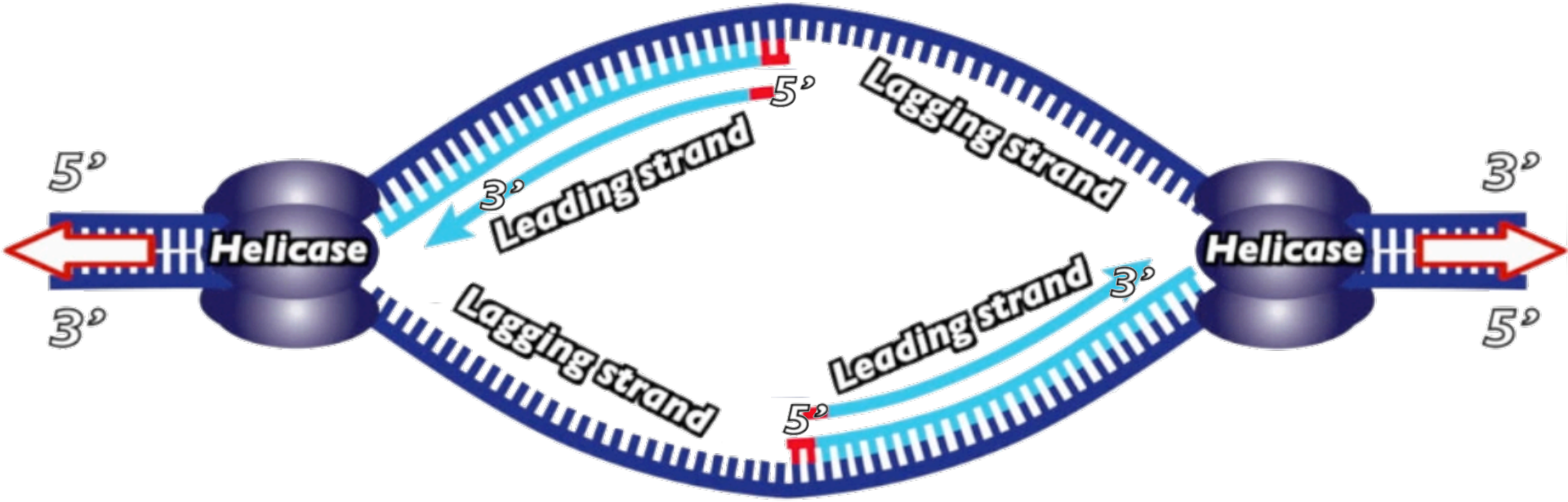


# Then DNA polymerase III takes over, adding new nucleotides at 3’ end

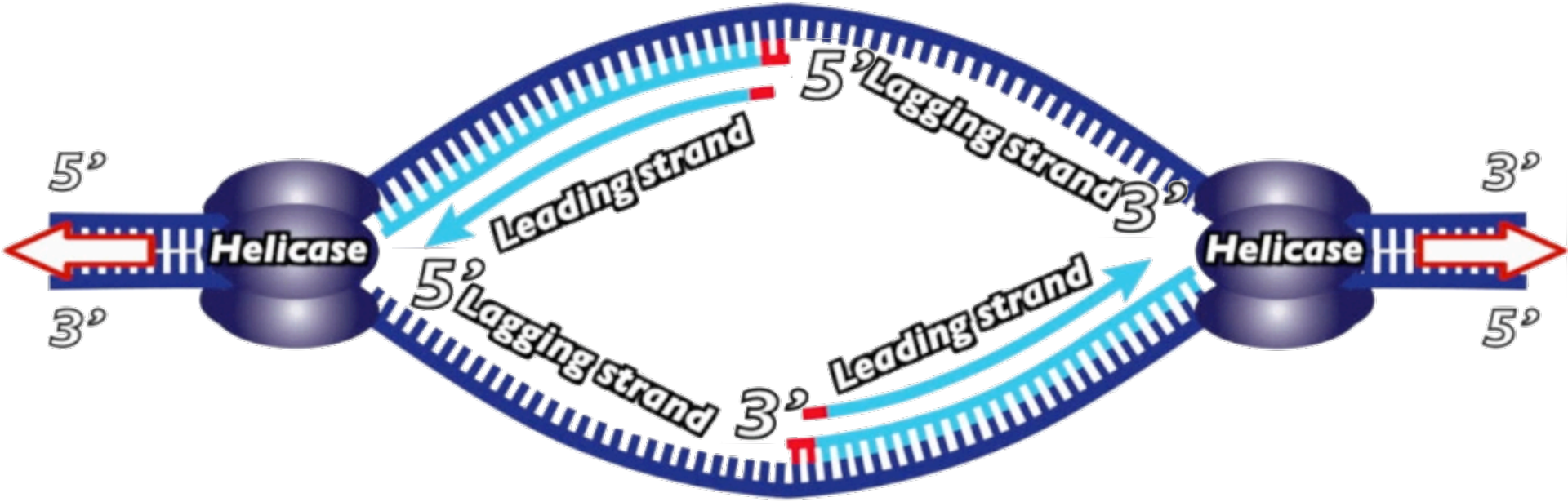


# Leading Strand: replication is continuous

- The strands where DNA polymerase III follows the opening replication fork is the *leading strand*
- ‚Replication moves *continuously in a 5' to 3' direction*.

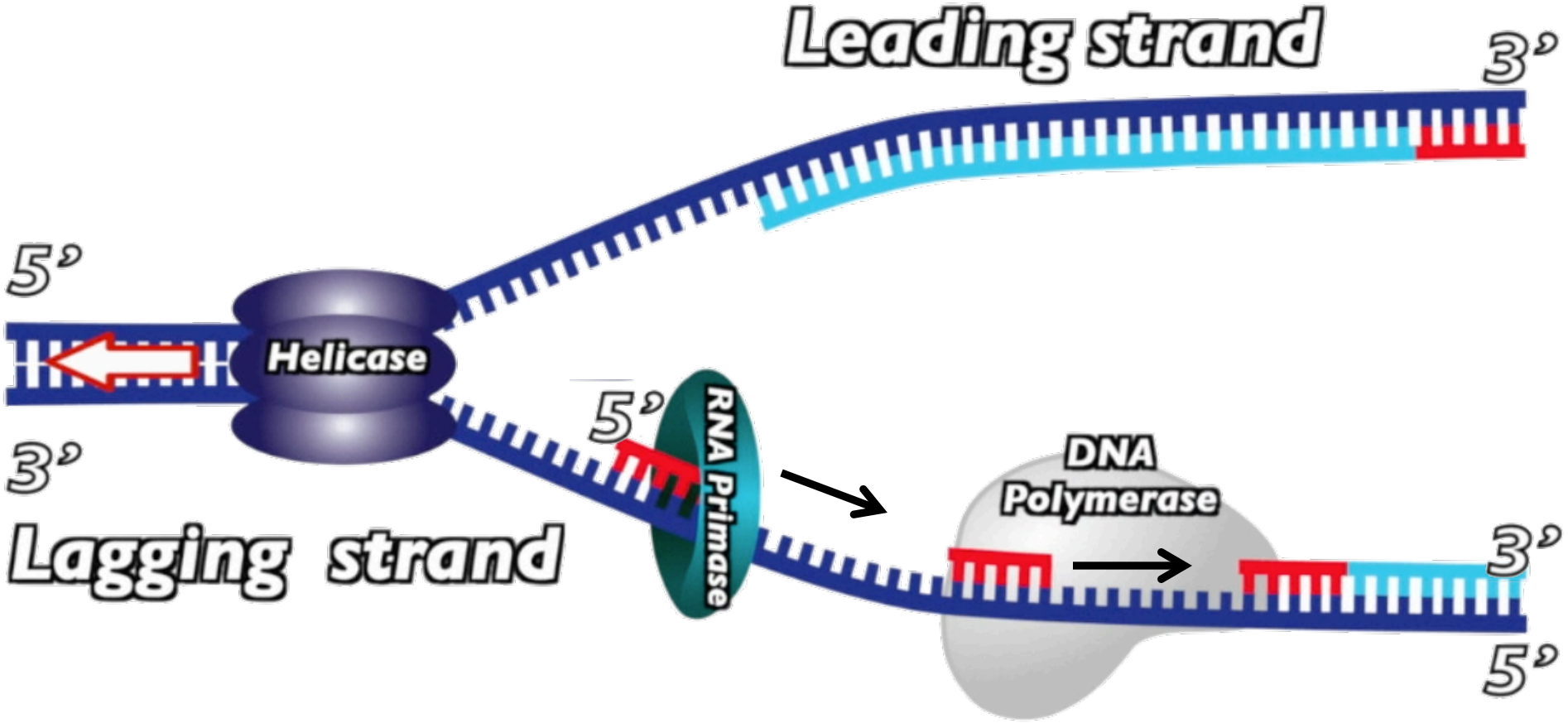


# Lagging Strand: how can we synthesize 3’ to 5’?

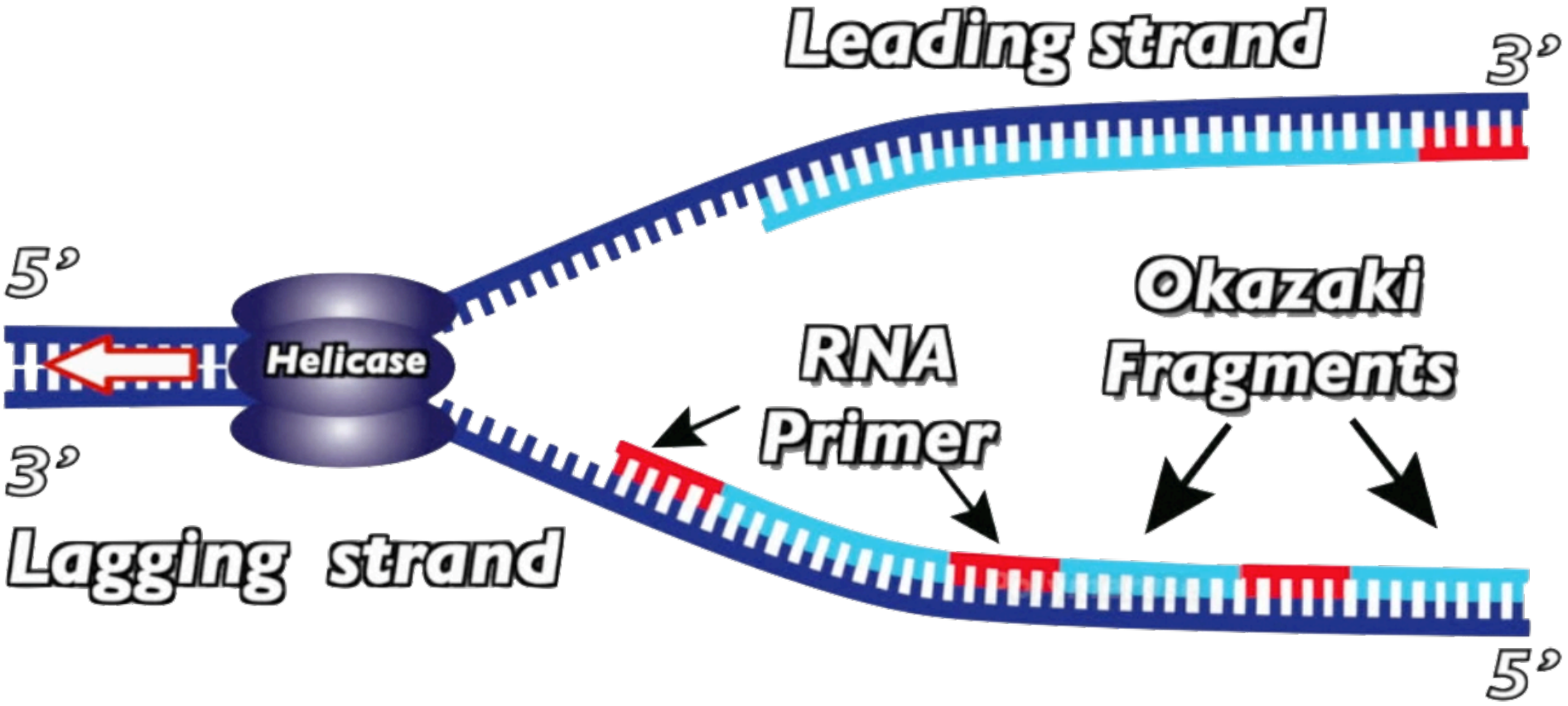


# Lagging Strand: synthesis is fragmentary

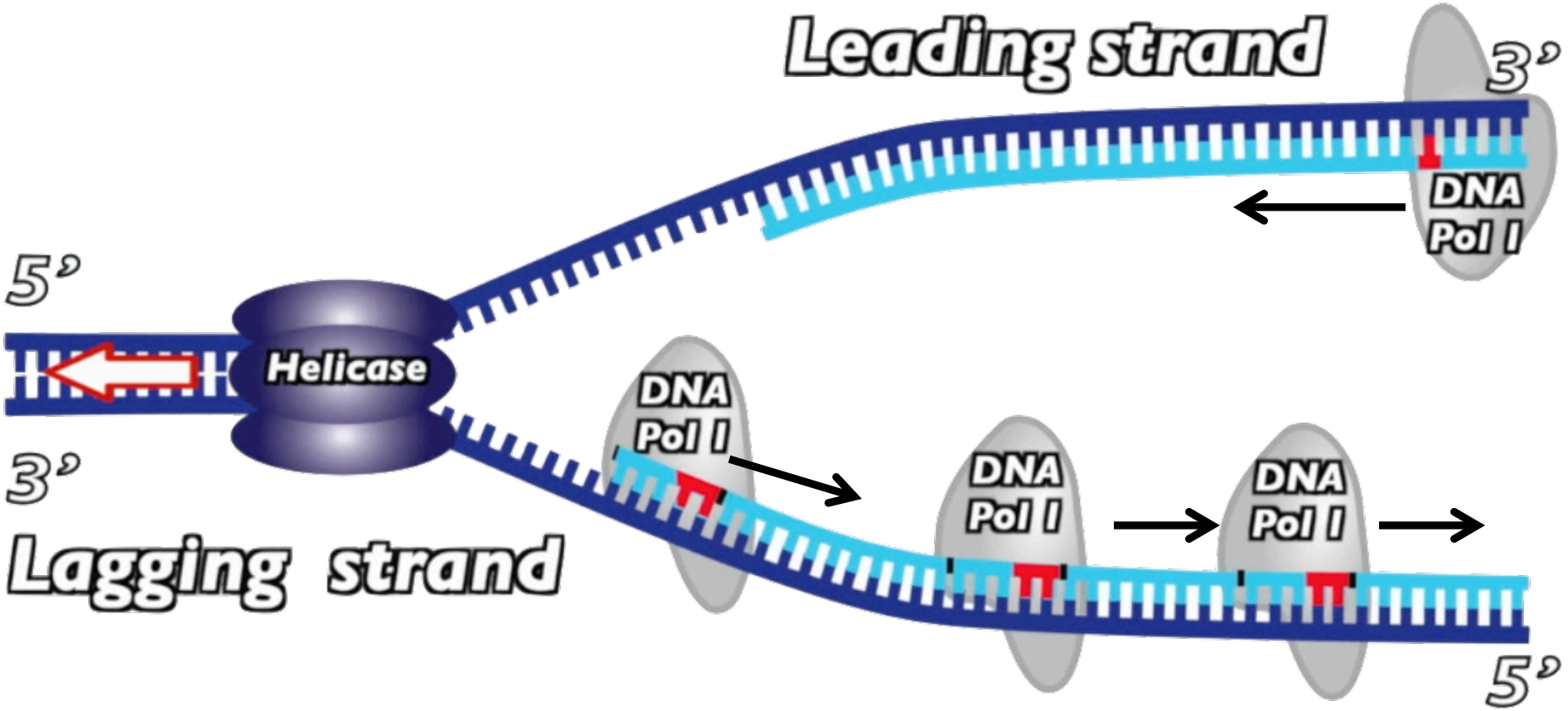
- In lagging strand, DNA polymerase III moves away from the opening replication fork.
- Replication is in short pieces called *Okazaki fragments*



# Okazaki Fragments

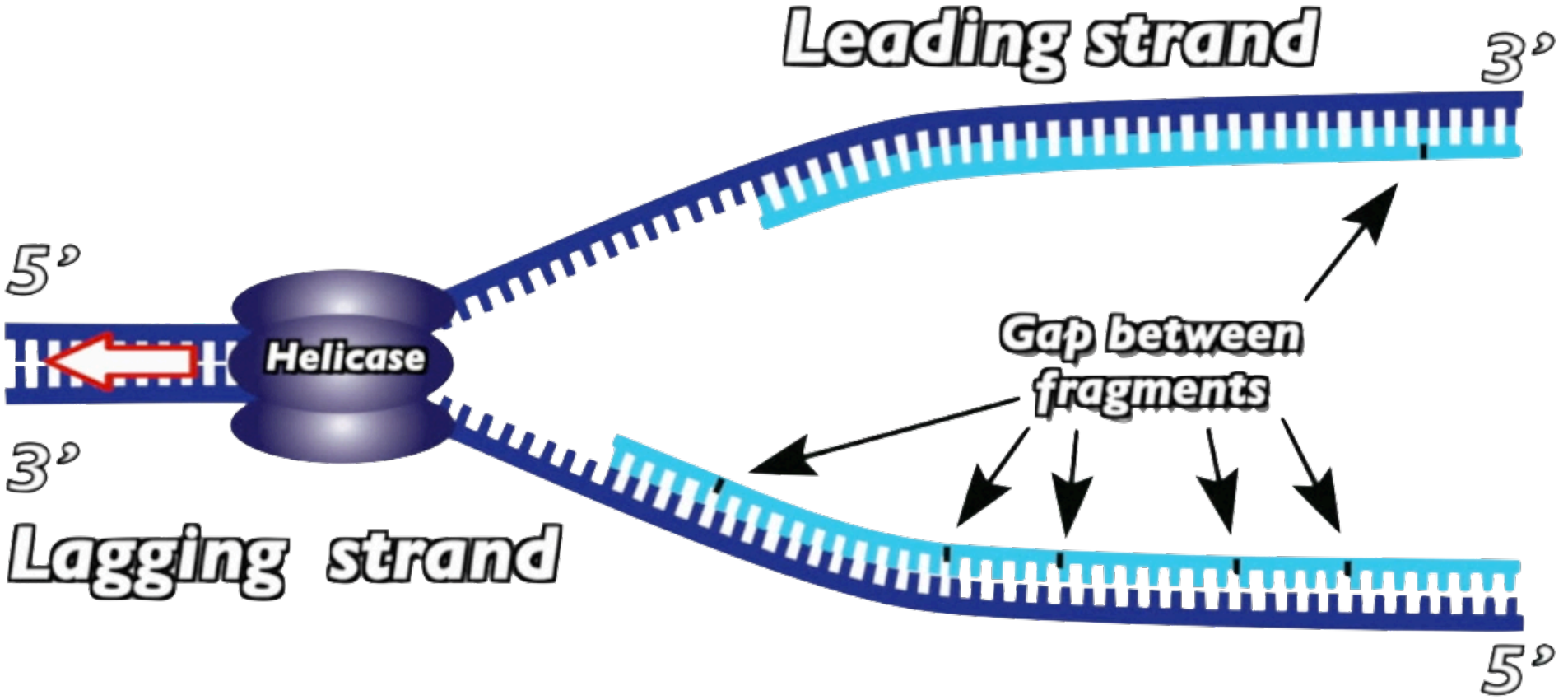


# DNA polymerase I removes the primers…

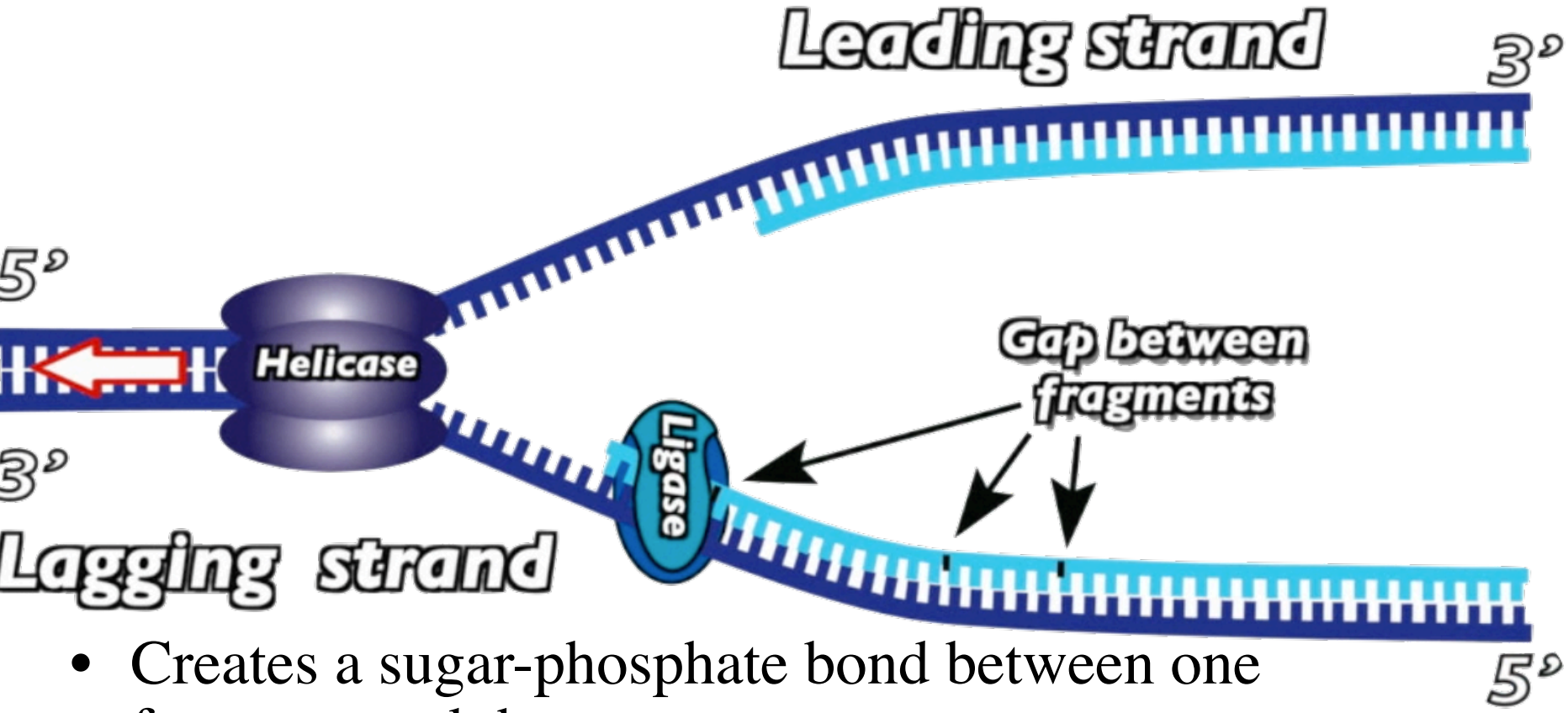



- And replaces the RNA with DNA.

# Fragmentary synthesis results in gaps in the sugar-phosphate backbone

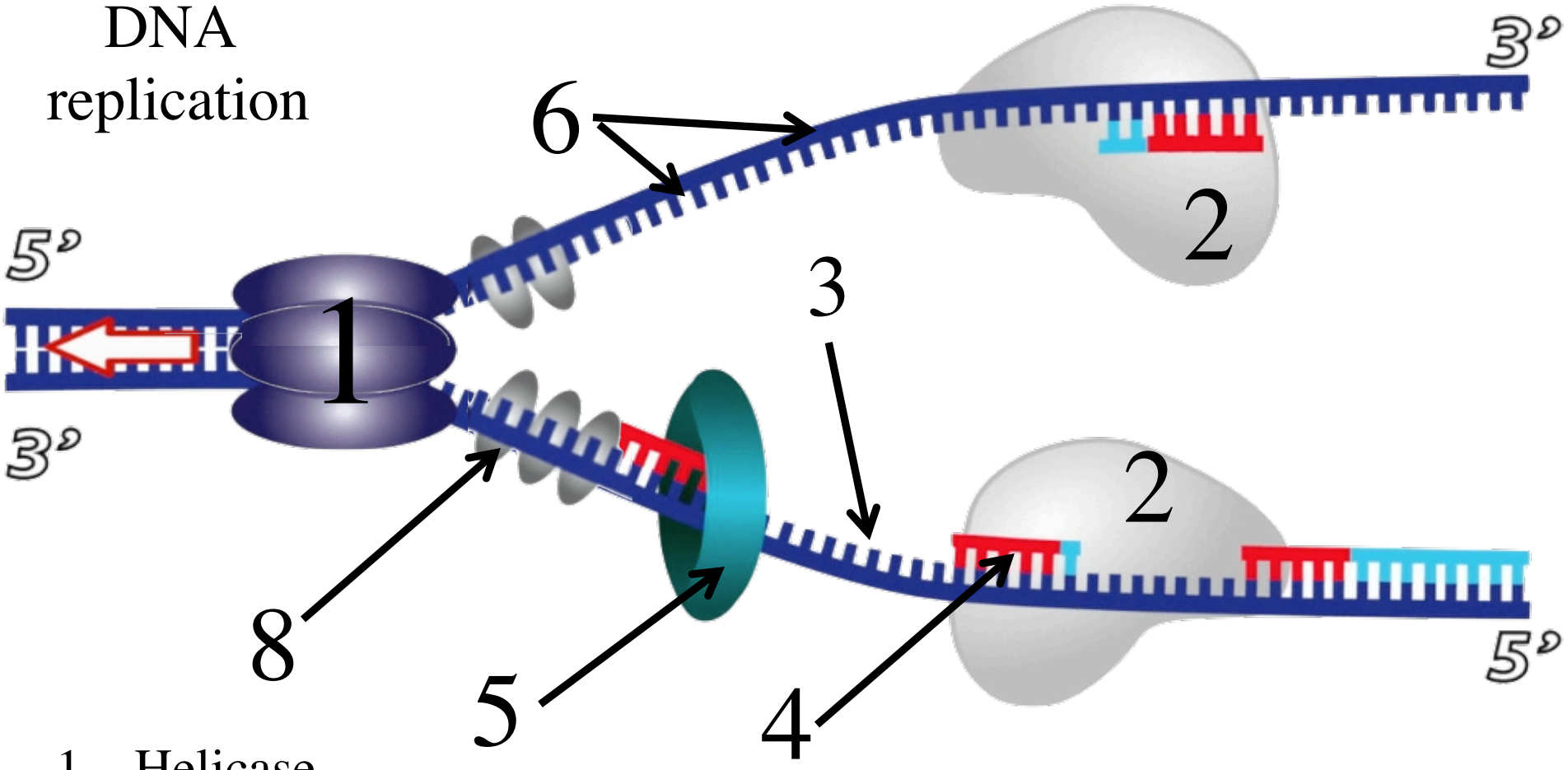


# …which are repaired by DNA ligase




- Creates a sugar-phosphate bond between one fragment and the next.

# DNA replication




1. Helicase
2. DNA polymerase
3. Template (lagging strand)
4. RNA Primer
5. Primase
6. Template (leading strand)
7. Single strand binding proteins

Date: 12/1. Number: 5-4. **DNA replication**

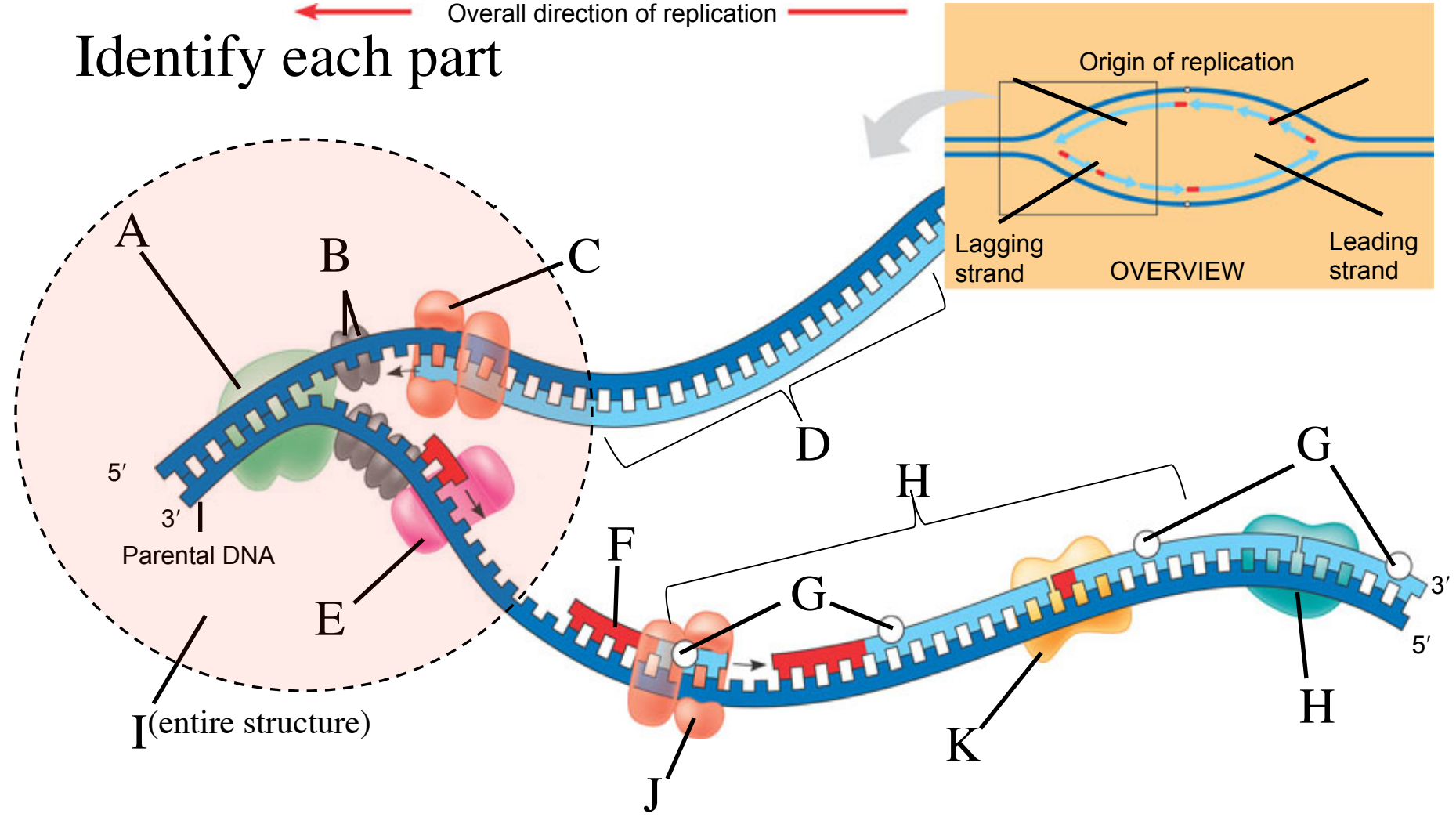
**OBJECTIVE: Describe DNA replication**

**HOMEWORK: FRQ: (see agenda) Imaginary interviews with Meselson/Stahl and Okazaki**

# CATALYST (copy and complete):

| ENZYME | FUNCTION |
|---|---|
| Helicase: | Separating the helix |
| DNA Polymerase: | Adding new nucleotides at 3' end |
| Primase: | Laying down an RNA Primer to start replication |
| Ligase | Connecting okazaki fragments |

# Identify each part

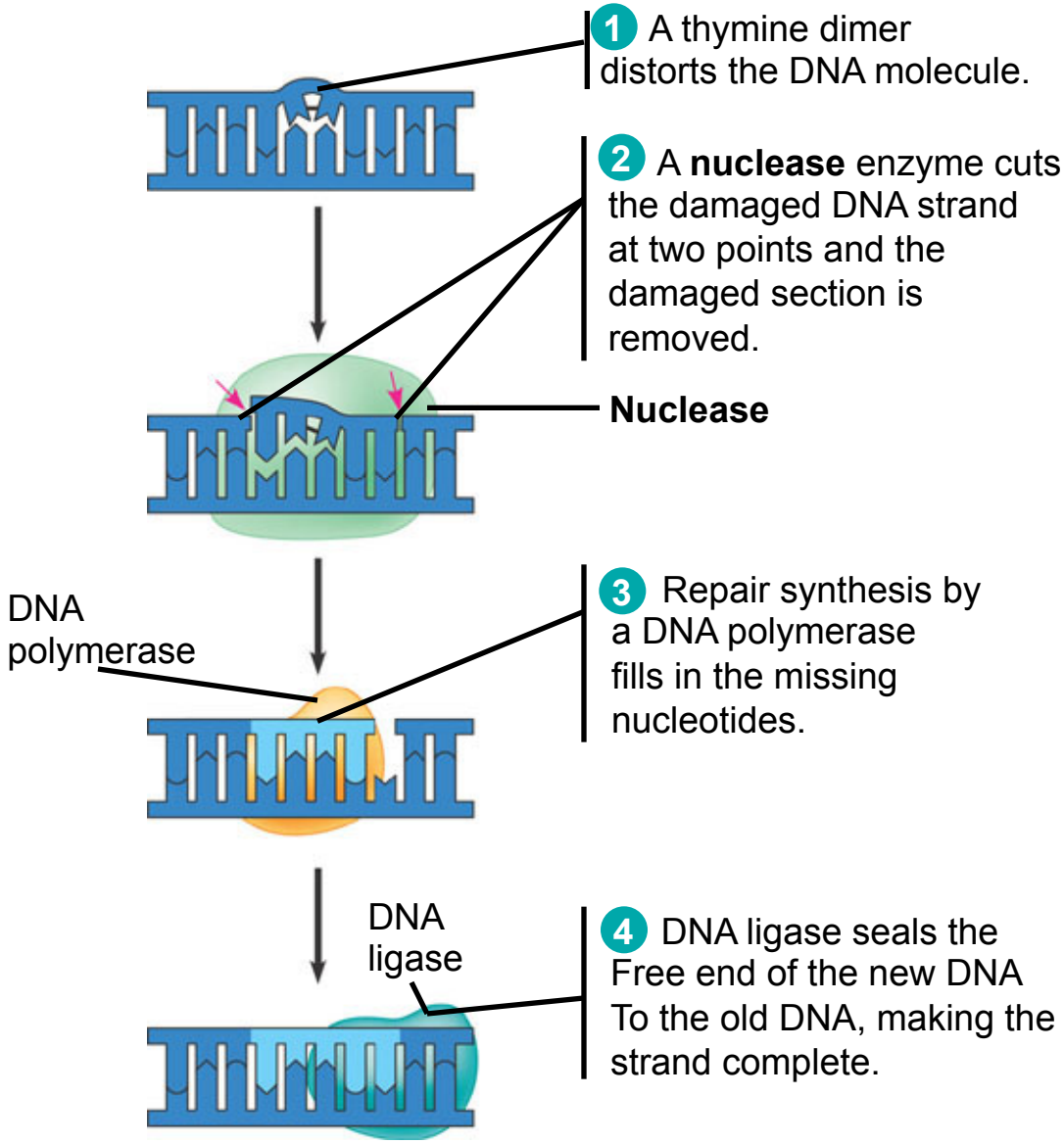



A. Helicase
B. SSBP
C. DNA Pol III
D. Leading strand
E. Primase

F. RNA primer
G. Okazaki fragments
H. Lagging strand
I. Replication fork
J. DNA poly III

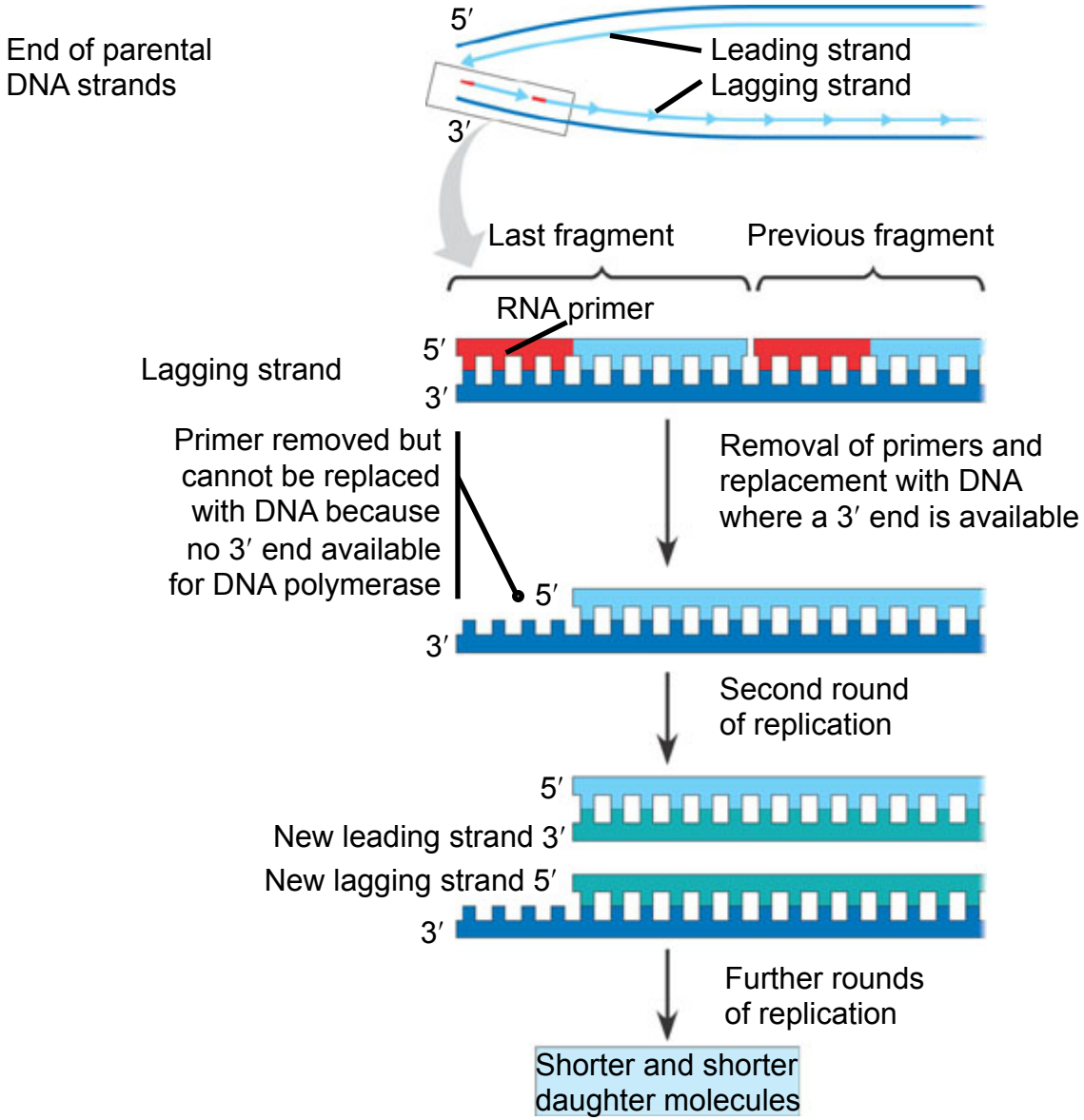
K. DNA pol I
H (right). Ligase

# Nucleotide excision repair of DNA damage

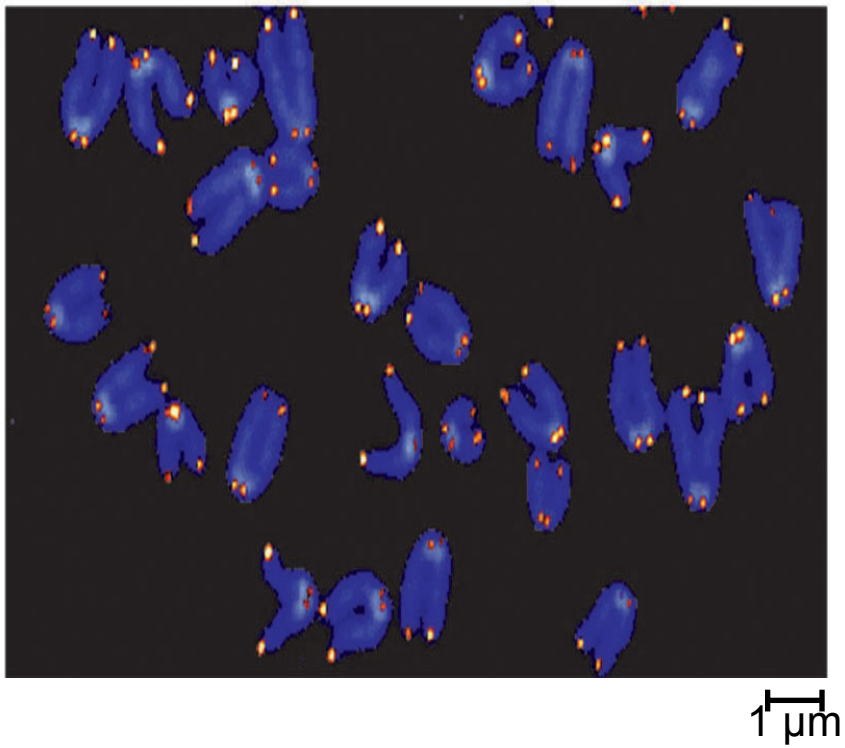



- System is inactivated in individuals with *xeroderma pigmentosum*.
- Sunlight's U.V. radiation leads to uncorrected mutations that go uncorrected.
- Result is skin cancer.

# The End Replication Problem in Linear DNA



# Telomeres




- Non-genetic multiple repeats of TTAGGG at the end of a chromosome.
- Telomerase lengthens telomeres. Contains RNA with AAUCCC, but
  - Is inactivated in body cells: only active in germ line cells that make sperm and eggs.
  - Is reactivated in cancer cells.